

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/00, 1/21, 15/63, 15/11, C07H 21/04, 21/02, C07K 14/00, A61K 38/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/13066</b> <b>(43) International Publication Date:</b> 18 March 1999 (18.03.99)
<b>(21) International Application Number:</b> PCT/US98/18724 <b>(22) International Filing Date:</b> 8 September 1998 (08.09.98) <b>(30) Priority Data:</b> 08/929,007 8 September 1997 (08.09.97) US <b>(71) Applicant:</b> GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). <b>(72) Inventors:</b> JACOBS, Kenneth; 151 Beaumont Avenue, Newton, MA 02160 (US). MCCOY, John, M.; 56 Howard Street, Reading, MA 01867 (US). LAVALLIE, Edward, R.; 113 Ann Lee Road, Harvard, MA 01451 (US). RACIE, Lisa, A.; 124 School Street, Acton, MA 01720 (US). EVANS, Cheryl; 111 Locust Street #41, Woburn, MA 01801 (US). MERBERG, David; 2 Orchard Drive, Acton, MA 01720 (US). TREACY, Maurice; 93 Walcott Road, Chestnut Hill, MA 02167 (US). AGOSTINO, Michael, J.; 26 Wolcott Avenue, Andover, MA 01810 (US). SPAULDING, Vikki; 11 Meadowbank Road, Billerica, MA 01821 (US). <b>(74) Agent:</b> SPRUNGER, Suzanne, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW. ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM		
<b>(57) Abstract</b>  Novel polynucleotides and proteins encoded by or related to SEQ ID NO:1 and SEQ ID NO:2 are disclosed.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM

5           This application is a continuation-in-part of application Ser. No. 60/093,045 (converted to a provisional application from non-provisional application Ser. No. 08/929,007), filed September 8, 1997, which is incorporated by reference herein.

FIELD OF THE INVENTION

10           The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins.

BACKGROUND OF THE INVENTION

15           Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered protein (i.e., partial DNA/amino acid sequence of the protein  
20 in the case of hybridization cloning; activity of the protein in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by making available large numbers of  
25 DNA/amino acid sequences for proteins that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the cell or tissue source in the case of PCR-based techniques. It is to these proteins and the polynucleotides encoding them that the present invention is directed.

30

35

40

### SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 69 to nucleotide 908;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 270 to nucleotide 908;
- 10 (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone bn97\_1 deposited under accession number ATCC 98535;
- (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone bn97\_1 deposited under accession number ATCC 98535;
- 15 (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone bn97\_1 deposited under accession number ATCC 98535;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone bn97\_1 deposited under accession number ATCC 98535;
- 20 (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:2;
- 25 (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and
- 30 (l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:1 from nucleotide 69 to nucleotide 908; the nucleotide sequence of SEQ ID NO:1 from nucleotide 270 to nucleotide 908; the nucleotide sequence of the full-length protein coding sequence of clone bn97\_1 deposited under accession number ATCC 98535; or the

nucleotide sequence of a mature protein coding sequence of clone bn97\_1 deposited under accession number ATCC 98535. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone bn97\_1 deposited under accession number ATCC 98535. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 83. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:2, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment comprising the amino acid sequence from amino acid 135 to amino acid 144 of SEQ ID NO:2.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:1.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- (b) the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 83;
- (c) fragments of the amino acid sequence of SEQ ID NO:2 comprising eight consecutive amino acids of SEQ ID NO:2; and
- (d) the amino acid sequence encoded by the cDNA insert of clone bn97\_1 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:2 or the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 83. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:2, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment comprising the amino acid sequence from amino acid 135 to amino acid 144 of SEQ ID NO:2.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3;
- 5 (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 562 to nucleotide 777;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 236 to nucleotide 673;
- (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone bn268\_11 deposited under accession number ATCC 98535;
- 10 (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone bn268\_11 deposited under accession number ATCC 98535;
- (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone bn268\_11 deposited under accession number ATCC 98535;
- 15 (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone bn268\_11 deposited under accession number ATCC 98535;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;
- 20 (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:4;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- 25 (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and
- (l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).

30 Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:3 from nucleotide 562 to nucleotide 777; the nucleotide sequence of SEQ ID NO:3 from nucleotide 236 to nucleotide 673; the nucleotide sequence of the full-length protein coding sequence of clone bn268\_11 deposited under accession number ATCC 98535; or the nucleotide sequence of a mature protein coding sequence of clone bn268\_11 deposited

under accession number ATCC 98535. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone bn268\_11 deposited under accession number ATCC 98535. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein  
5 comprising the amino acid sequence of SEQ ID NO:4 from amino acid 1 to amino acid 37. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:4, or a  
10 polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment comprising the amino acid sequence from amino acid 31 to amino acid 40 of SEQ ID NO:4.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:3.

15 In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:4;
- (b) the amino acid sequence of SEQ ID NO:4 from amino acid 1 to  
20 amino acid 37;
- (c) fragments of the amino acid sequence of SEQ ID NO:4 comprising eight consecutive amino acids of SEQ ID NO:4; and
- (d) the amino acid sequence encoded by the cDNA insert of clone bn268\_11 deposited under accession number ATCC 98535;

25 the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:4 or the amino acid sequence of SEQ ID NO:4 from amino acid 1 to amino acid 37. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment preferably comprising  
30 eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:4, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment comprising the amino acid sequence from amino acid 31 to amino acid 40 of SEQ ID NO:4.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;
- 5 (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 286 to nucleotide 1686;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 544 to nucleotide 1686;
- (d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 365 to nucleotide 1160;
- 10 (e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone cb96\_10 deposited under accession number ATCC 98535;
- (f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone cb96\_10 deposited under accession number ATCC 98535;
- 15 (g) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone cb96\_10 deposited under accession number ATCC 98535;
- (h) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone cb96\_10 deposited under accession number ATCC 98535;
- 20 (i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;
- (j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:6;
- 25 (k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;
- (l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and
- 30 (m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(j).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:5 from nucleotide 286 to nucleotide 1686; the nucleotide sequence of SEQ ID NO:5 from nucleotide 544 to nucleotide 1686; the nucleotide sequence of SEQ ID NO:5 from



nucleotide 365 to nucleotide 1160; the nucleotide sequence of the full-length protein coding sequence of clone cb96\_10 deposited under accession number ATCC 98535; or the nucleotide sequence of a mature protein coding sequence of clone cb96\_10 deposited under accession number ATCC 98535. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone cb96\_10 deposited under accession number ATCC 98535. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6 from amino acid 28 to amino acid 395. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:6, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment comprising the amino acid sequence from amino acid 228 to amino acid 237 of SEQ ID NO:6.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:5.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6;
- (b) the amino acid sequence of SEQ ID NO:6 from amino acid 28 to amino acid 395;
- (c) fragments of the amino acid sequence of SEQ ID NO:6 comprising eight consecutive amino acids of SEQ ID NO:6; and
- (d) the amino acid sequence encoded by the cDNA insert of clone cb96\_10 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:6 or the amino acid sequence of SEQ ID NO:6 from amino acid 28 to amino acid 395. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:6, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6

having biological activity, the fragment comprising the amino acid sequence from amino acid 228 to amino acid 237 of SEQ ID NO:6.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 5           (a)    a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7;
- (b)    a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 99 to nucleotide 1049;
- (c)    a polynucleotide comprising the nucleotide sequence of SEQ ID  
10       NO:7 from nucleotide 222 to nucleotide 1049;
- (d)    a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 632 to nucleotide 998;
- (e)    a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone cb213\_11 deposited under accession  
15       number ATCC 98535;
- (f)    a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone cb213\_11 deposited under accession number ATCC 98535;
- (g)    a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone cb213\_11 deposited under accession number  
20       ATCC 98535;
- (h)    a polynucleotide encoding a mature protein encoded by the cDNA insert of clone cb213\_11 deposited under accession number ATCC 98535;
- (i)    a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;
- 25       (j)    a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:8;
- (k)    a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;
- 30       (l)    a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and
- (m)    a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(j).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:7 from nucleotide 99 to nucleotide 1049; the nucleotide sequence of SEQ ID NO:7 from nucleotide 222 to nucleotide 1049; the nucleotide sequence of SEQ ID NO:7 from nucleotide 632 to nucleotide 998; the nucleotide sequence of the full-length protein coding sequence of clone cb213\_11 deposited under accession number ATCC 98535; or the nucleotide sequence of a mature protein coding sequence of clone cb213\_11 deposited under accession number ATCC 98535. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone cb213\_11 deposited under accession number ATCC 98535. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8 from amino acid 187 to amino acid 300. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:8, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment comprising the amino acid sequence from amino acid 153 to amino acid 162 of SEQ ID NO:8.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:7.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:8;
- (b) the amino acid sequence of SEQ ID NO:8 from amino acid 187 to amino acid 300;
- (c) fragments of the amino acid sequence of SEQ ID NO:8 comprising eight consecutive amino acids of SEQ ID NO:8; and
- (d) the amino acid sequence encoded by the cDNA insert of clone cb213\_11 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:8 or the amino acid sequence of SEQ ID NO:8 from amino acid 187 to amino acid 300. In further preferred embodiments, the present invention provides a protein comprising a fragment of the

amino acid sequence of SEQ ID NO:8 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:8, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment comprising the amino acid  
5 sequence from amino acid 153 to amino acid 162 of SEQ ID NO:8.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;
- 10 (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 3003 to nucleotide 3137;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 3072 to nucleotide 3137;
- (d) a polynucleotide comprising the nucleotide sequence of SEQ ID  
15 NO:9 from nucleotide 2713 to nucleotide 3114;
- (e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone cj457\_4 deposited under accession number ATCC 98535;
- (f) a polynucleotide encoding the full-length protein encoded by the  
20 cDNA insert of clone cj457\_4 deposited under accession number ATCC 98535;
- (g) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone cj457\_4 deposited under accession number ATCC 98535;
- (h) a polynucleotide encoding a mature protein encoded by the cDNA  
25 insert of clone cj457\_4 deposited under accession number ATCC 98535;
- (i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;
- (j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment  
30 comprising eight consecutive amino acids of SEQ ID NO:10;
- (k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;
- (l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(j).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:9 from nucleotide 3003 to nucleotide 3137; the nucleotide sequence of SEQ ID NO:9  
5 from nucleotide 3072 to nucleotide 3137; the nucleotide sequence of SEQ ID NO:9 from nucleotide 2713 to nucleotide 3114; the nucleotide sequence of the full-length protein coding sequence of clone cj457\_4 deposited under accession number ATCC 98535; or the nucleotide sequence of a mature protein coding sequence of clone cj457\_4 deposited under accession number ATCC 98535. In other preferred embodiments, the polynucleotide  
10 encodes the full-length or a mature protein encoded by the cDNA insert of clone cj457\_4 deposited under accession number ATCC 98535. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 37. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein  
15 comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:10, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment comprising the amino acid sequence from amino acid 17  
20 to amino acid 26 of SEQ ID NO:10.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:9.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group  
25 consisting of:

- (a) the amino acid sequence of SEQ ID NO:10;
- (b) the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 37;
- (c) fragments of the amino acid sequence of SEQ ID NO:10 comprising  
30 eight consecutive amino acids of SEQ ID NO:10; and
- (d) the amino acid sequence encoded by the cDNA insert of clone cj457\_4 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:10 or the amino acid sequence

of SEQ ID NO:10 from amino acid 1 to amino acid 37. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID  
5 NO:10, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment comprising the amino acid sequence from amino acid 17 to amino acid 26 of SEQ ID NO:10.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 10 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 284 to nucleotide 1357;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID  
15 NO:11 from nucleotide 603 to nucleotide 1233;
- (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone cz653\_11 deposited under accession number ATCC 98535;
- (e) a polynucleotide encoding the full-length protein encoded by the  
20 cDNA insert of clone cz653\_11 deposited under accession number ATCC 98535;
- (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone cz653\_11 deposited under accession number ATCC 98535;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA  
25 insert of clone cz653\_11 deposited under accession number ATCC 98535;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment  
30 comprising eight consecutive amino acids of SEQ ID NO:12;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:11 from nucleotide 284 to nucleotide 1357; the nucleotide sequence of SEQ ID NO:11 from nucleotide 603 to nucleotide 1233; the nucleotide sequence of the full-length protein coding sequence of clone cz653\_11 deposited under accession number ATCC 98535; or the nucleotide sequence of a mature protein coding sequence of clone cz653\_11 deposited under accession number ATCC 98535. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone cz653\_11 deposited under accession number ATCC 98535. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12 from amino acid 147 to amino acid 358. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:12, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment comprising the amino acid sequence from amino acid 174 to amino acid 183 of SEQ ID NO:12.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:11.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:12;
- (b) the amino acid sequence of SEQ ID NO:12 from amino acid 147 to amino acid 358;
- (c) fragments of the amino acid sequence of SEQ ID NO:12 comprising eight consecutive amino acids of SEQ ID NO:12; and
- (d) the amino acid sequence encoded by the cDNA insert of clone cz653\_11 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:12 or the amino acid sequence of SEQ ID NO:12 from amino acid 147 to amino acid 358. In further preferred

embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:12, or a protein comprising a fragment of the amino acid sequence of  
5 SEQ ID NO:12 having biological activity, the fragment comprising the amino acid sequence from amino acid 174 to amino acid 183 of SEQ ID NO:12.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 10 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 621 to nucleotide 1763;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 1461 to nucleotide 1763;
- 15 (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone dx138\_4 deposited under accession number ATCC 98535;
- (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone dx138\_4 deposited under accession number ATCC 98535;
- 20 (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone dx138\_4 deposited under accession number ATCC 98535;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone dx138\_4 deposited under accession number ATCC 98535;
- 25 (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:14;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:14;
- 30 (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and



(l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:13 from nucleotide 621 to nucleotide 1763; the nucleotide sequence of SEQ ID NO:13 from nucleotide 1461 to nucleotide 1763; the nucleotide sequence of the full-length protein coding sequence of clone dx138\_4 deposited under accession number ATCC 98535; or the nucleotide sequence of a mature protein coding sequence of clone dx138\_4 deposited under accession number ATCC 98535. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone dx138\_4 deposited under accession number ATCC 98535. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:14 from amino acid 83 to amino acid 229. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:14, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity, the fragment comprising the amino acid sequence from amino acid 185 to amino acid 194 of SEQ ID NO:14.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:13.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:14;
- (b) the amino acid sequence of SEQ ID NO:14 from amino acid 83 to amino acid 229;
- (c) fragments of the amino acid sequence of SEQ ID NO:14 comprising eight consecutive amino acids of SEQ ID NO:14; and
- (d) the amino acid sequence encoded by the cDNA insert of clone dx138\_4 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:14 or the amino acid sequence of SEQ ID NO:14 from amino acid 83 to amino acid 229. In further preferred

embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:14, or a protein comprising a fragment of the amino acid sequence of  
5 SEQ ID NO:14 having biological activity, the fragment comprising the amino acid sequence from amino acid 185 to amino acid 194 of SEQ ID NO:14.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- 10 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from nucleotide 119 to nucleotide 295;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from nucleotide 191 to nucleotide 295;
- 15 (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone ij167\_5 deposited under accession number ATCC 98535;
- (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone ij167\_5 deposited under accession number ATCC 98535;
- 20 (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone ij167\_5 deposited under accession number ATCC 98535;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone ij167\_5 deposited under accession number ATCC 98535;
- 25 (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:16;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:16;
- 30 (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and

(l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:15 from nucleotide 119 to nucleotide 295; the nucleotide sequence of SEQ ID NO:15 from nucleotide 191 to nucleotide 295; the nucleotide sequence of the full-length protein coding sequence of clone ij167\_5 deposited under accession number ATCC 98535; or the nucleotide sequence of a mature protein coding sequence of clone ij167\_5 deposited under accession number ATCC 98535. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone ij167\_5 deposited under accession number ATCC 98535. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:16 from amino acid 1 to amino acid 26. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:16, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 having biological activity, the fragment comprising the amino acid sequence from amino acid 24 to amino acid 33 of SEQ ID NO:16.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:15.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:16;
- (b) the amino acid sequence of SEQ ID NO:16 from amino acid 1 to amino acid 26;
- (c) fragments of the amino acid sequence of SEQ ID NO:16 comprising eight consecutive amino acids of SEQ ID NO:16; and
- (d) the amino acid sequence encoded by the cDNA insert of clone ij167\_5 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:16 or the amino acid sequence of SEQ ID NO:16 from amino acid 1 to amino acid 26. In further preferred embodiments,

the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) consecutive amino acids of SEQ ID NO:16, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 having biological activity, the fragment comprising the amino acid sequence from amino acid 24 to amino acid 33 of SEQ ID NO:16.

In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with such polynucleotide compositions. Also provided by the present invention are organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein.

Processes are also provided for producing a protein, which comprise:

- (a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium; and
- (b) purifying the protein from the culture.

The protein produced according to such methods is also provided by the present invention.

Protein compositions of the present invention may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are schematic representations of the pED6 and pNOTs vectors, respectively, used for deposit of clones disclosed herein.

30

#### DETAILED DESCRIPTION

##### ISOLATED PROTEINS AND POLYNUCLEOTIDES

Nucleotide and amino acid sequences, as presently determined, are reported below for each clone and protein disclosed in the present application. The nucleotide

sequence of each clone can readily be determined by sequencing of the deposited clone in accordance with known methods. The predicted amino acid sequence (both full-length and mature forms) can then be determined from such nucleotide sequence. The amino acid sequence of the protein encoded by a particular clone can also be determined by  
5 expression of the clone in a suitable host cell, collecting the protein and determining its sequence. For each disclosed protein applicants have identified what they have determined to be the reading frame best identifiable with sequence information available at the time of filing.

As used herein a "secreted" protein is one which, when expressed in a suitable host  
10 cell, is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g., receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins which are transported across the membrane of the endoplasmic reticulum.

15

#### Clone "bn97\_1"

A polynucleotide of the present invention has been identified as clone "bn97\_1". bn97\_1 was isolated from a human adult placenta cDNA library was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the  
20 amino acid sequence of the encoded protein. bn97\_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "bn97\_1 protein").

The nucleotide sequence of bn97\_1 as presently determined is reported in SEQ ID NO:1. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the bn97\_1 protein corresponding to the foregoing nucleotide  
25 sequence is reported in SEQ ID NO:2. Amino acids 55 to 67 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 68, or are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone bn97\_1 should be approximately 1700 bp.

30 The nucleotide sequence disclosed herein for bn97\_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. bn97\_1 demonstrated at least some identity with sequences identified as AA046689 (zk72h06.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 488411 3'), D30934 (Human fetal-lung cDNA 5'-end sequence), R78820 (yi90b03.r1

Homo sapiens cDNA clone 146477 5'), and R91687 (yq10h09.s1 Homo sapiens cDNA clone 196577 3'). The predicted amino acid sequence disclosed herein for bn97\_1 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted bn97\_1 protein demonstrated at least some identity with sequences identified as A10431 (Hepatitis-B virus surface antigen P31). The bn97\_1 protein also shows some identity (30% identity, 50% conserved amino acids) to both bovine and human lectin-like receptor for oxidized LDL (low-density lipoprotein). While this homology is weak, it gets stronger (44% identity and 62% conserved amino acids) in the lectin-like domain. Further, the 3' untranslated region of the bovine receptor has seven mRNA unstabilising sequences (ATTTA) and bn97\_1 has four in its 3' untranslated region. This lectin-like receptor for oxidized LDL (designated LOX-1, Sawamura *et al.*, 1997, *Nature* 386: 73-77) is an integral membrane protein which binds oxidized low-density lipoproteins, internalizes them into the endothelial cells and destroys them, thus playing a crucial role in the pathogenesis of atherosclerosis. Based upon identity, bn97\_1 proteins and each identical protein or peptide may share at least some activity.

#### Clone "bn268\_11"

A polynucleotide of the present invention has been identified as clone "bn268\_11". bn268\_11 was isolated from a human adult placenta cDNA library was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. bn268\_11 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "bn268\_11 protein").

The nucleotide sequence of bn268\_11 as presently determined is reported in SEQ ID NO:3. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the bn268\_11 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:4.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone bn268\_11 should be approximately 1050 bp.

The nucleotide sequence disclosed herein for bn268\_11 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. bn268\_11 demonstrated at least some identity with sequences

identified as D62832 (Human aorta cDNA 5'-end GEN-330C09) and U20159 (Mus musculus 76 kDa tyrosine phosphoprotein SLP-76 mRNA, complete cds). The predicted amino acid sequence disclosed herein for bn268\_11 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The  
5 predicted bn268\_11 protein demonstrated at least some identity with sequences identified as D83171 (GDP-GTP exchange protein for Rho1p [Saccharomyces cerevisiae]). Based upon identity, bn268\_11 proteins and each identical protein or peptide may share at least some activity. The TopPredII computer program predicts a potential transmembrane domain within the bn268\_11 protein sequence centered around amino acid 33 of SEQ ID  
10 NO:4; this region may also function as a signal sequence.

#### Clone "cb96\_10"

A polynucleotide of the present invention has been identified as clone "cb96\_10". cb96\_10 was isolated from a human fetal brain cDNA library was identified as encoding  
15 a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. cb96\_10 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "cb96\_10 protein").

The nucleotide sequence of cb96\_10 as presently determined is reported in SEQ ID NO:5. What applicants presently believe to be the proper reading frame and the  
20 predicted amino acid sequence of the cb96\_10 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:6. Amino acids 74 to 86 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 87, or are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone  
25 cb96\_10 should be approximately 2100 bp.

The nucleotide sequence disclosed herein for cb96\_10 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. cb96\_10 demonstrated at least some identity with sequences identified as AA459012, AA459236, AA256744 (zs31h11.r1 Soares NbHTGBC Homo  
30 sapiens cDNA clone 686853 5'), N54489 (yv40f07.s1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone 245221 3'), and N57339 (yw81h07.r1 Homo sapiens cDNA clone 258685 5'). The predicted amino acid sequence disclosed herein for cb96\_10 was searched against the GenPept and GeneSeq amino acid sequence databases using the

BLASTX search protocol. The predicted cb96\_10 protein demonstrated at least some identity with sequences identified as X80036 (ascorbate peroxidase [Arabidopsis thaliana]). Based upon identity, cb96\_10 proteins and each identical protein or peptide may share at least some activity. The TopPredII computer program predicts seven  
5 potential transmembrane domains within the cb96\_10 protein sequence, centered around amino acid residues 25, 80, 125, 225, 300, 350, and 440 of SEQ ID NO:6. Therefore, cb96\_10 is likely to be an integral membrane protein with multiple helices in the membrane; it also contains the sequence motif of the actinin-type actin-binding domains that are believed to anchor actin to the cell membrane.

10

#### Clone "cb213\_11"

A polynucleotide of the present invention has been identified as clone "cb213\_11". cb213\_11 was isolated from a human fetal brain cDNA library was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid  
15 sequence of the encoded protein. cb213\_11 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "cb213\_11 protein").

The nucleotide sequence of cb213\_11 as presently determined is reported in SEQ ID NO:7. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the cb213\_11 protein corresponding to the foregoing  
20 nucleotide sequence is reported in SEQ ID NO:8. Amino acids 29 to 41 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 42, or are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone cb213\_11 should be approximately 2400 bp.

25 The nucleotide sequence disclosed herein for cb213\_11 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. cb213\_11 demonstrated at least some identity with sequences identified as AA332165 (EST36344 Embryo, 8 week I Homo sapiens cDNA 5' end) and R34507 (g58a03.r1 Homo sapiens cDNA clone 36801 5'). The predicted amino acid  
30 sequence disclosed herein for cb213\_11 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted cb213\_11 protein demonstrated at least some identity with sequences identified as U39847



(AO13 ankyrin [Caenorhabditis elegans]). Based upon identity, cb213\_11 proteins and each identical protein or peptide may share at least some activity.

Clone "cj457\_4"

5 A polynucleotide of the present invention has been identified as clone "cj457\_4". cj457\_4 was isolated from a human fetal brain cDNA library was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. cj457\_4 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "cj457\_4 protein").

10 The nucleotide sequence of cj457\_4 as presently determined is reported in SEQ ID NO:9. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the cj457\_4 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:10. Amino acids 11 to 23 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 24, or  
15 are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone cj457\_4 should be approximately 3350 bp.

The nucleotide sequence disclosed herein for cj457\_4 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and  
20 FASTA search protocols. cj457\_4 demonstrated at least some identity with sequences identified as T92881 (ye22a10.s1 Homo sapiens cDNA clone 118458 3') and T92488 (ye21g09.r1 Homo sapiens cDNA clone 118432 5'). Based upon identity, cj457\_4 proteins and each identical protein or peptide may share at least some activity. The TopPredII computer program predicts a potential transmembrane domain within the cj457\_4 protein  
25 sequence, centered around amino acid 17 of SEQ ID NO:10; this region may also function as a signal sequence.

Clone "cz653\_11"

A polynucleotide of the present invention has been identified as clone "cz653\_11".  
30 cz653\_11 was isolated from a human adult testes cDNA library was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. cz653\_11 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "cz653\_11 protein").

The nucleotide sequence of cz653\_11 as presently determined is reported in SEQ ID NO:11... What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the cz653\_11 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:12.

- 5           The EcoRI/NotI restriction fragment obtainable from the deposit containing clone cz653\_11 should be approximately 1300 bp.

The nucleotide sequence disclosed herein for cz653\_11 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. cz653\_11 demonstrated at least some identity with sequences  
10 identified as AA024740 (ze76c09.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 364912 3'), AA203204 (zx57b04.r1 Soares fetal liver spleen 1NFLS S1 Homo sapiens cDNA clone 446575 5' similar to contains element MSR1 repetitive element), W72894 (zd59e06.s1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 344962 3'), and W76099 (zd59e06.r1 Soares fetal heart NbHH19W Homo sapiens cDNA clone  
15 344962 5'). The predicted cz653\_11 demonstrated similarity to various WD-40 repeat containing proteins such as beta transducin-like protein (L28125) and coatomer, beta-prime subunit (AJ006523). The homology appears to be due to the presence of the Beta-transducin family Trp-Asp repeats signature (WD-40) beginning at residue 262 of SEQ ID NO:12. The WD-40 repeat has been thought to be a protein-protein interaction  
20 domain. Based upon identity, cz653\_11 proteins and each identical protein or peptide may share at least some activity. The TopPredII computer program predicts a potential transmembrane domain within the cz653\_11 protein sequence centered around amino acid 200 of SEQ ID NO:12.

25           Clone "dx138\_4"

A polynucleotide of the present invention has been identified as clone "dx138\_4". dx138\_4 was isolated from a human adult testes cDNA library was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. dx138\_4 is a full-length clone, including the entire  
30 coding sequence of a secreted protein (also referred to herein as "dx138\_4 protein").

The nucleotide sequence of dx138\_4 as presently determined is reported in SEQ ID NO:13. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the dx138\_4 protein corresponding to the foregoing

nucleotide sequence is reported in SEQ ID NO:14. Amino acids 268 to 280 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 281, or are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone  
5 dx138\_4 should be approximately 2300 bp.

The nucleotide sequence disclosed herein for dx138\_4 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. dx138\_4 demonstrated at least some identity with sequences identified as AA108970 (ml63a06.r1 Stratagene mouse testis (#937308) Mus musculus  
10 cDNA clone 516658 5'), AA280976 (zs97f01.r1 Soares NbHTGBC Homo sapiens cDNA clone 711577 5' similar to contains Alu repetitive element), H99316 (yx23a03.s1 Homo sapiens cDNA clone 262540 3'), T36050 (EST96120 Homo sapiens cDNA 5'), X85637 (H.sapiens mRNA for expressed sequence tag, clone CAM tEST417 (A)), and Z22280 (H.sapiens DNA sequence). Based upon identity, dx138\_4 proteins and each identical  
15 protein or peptide may share at least some activity.

#### Clone "ij167\_5"

A polynucleotide of the present invention has been identified as clone "ij167\_5". ij167\_5 was isolated from a human adult blood (peripheral blood mononuclear cells treated  
20 *in vivo* with G-CSF) cDNA library was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. ij167\_5 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "ij167\_5 protein").

The nucleotide sequence of ij167\_5 as presently determined is reported in SEQ ID  
25 NO:15. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the ij167\_5 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:16. Amino acids 12 to 24 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 25, or are a transmembrane domain.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone  
30 ij167\_5 should be approximately 1050 bp.

The nucleotide sequence disclosed herein for ij167\_5 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and

FASTA search protocols. ij167\_5 demonstrated at least some identity with sequences identified as N71115 (za87h10.s1 Homo sapiens cDNA clone 299587 3'), T85491 (yd78b01.r1 Homo sapiens cDNA clone 114313 5'), W04374 (za43f06.r1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone 295331 5'), W05476 (za87h10.r1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 299587 5'), and W40146 (zb74d09.r1 Soares fetal lung NbHL19W Homo sapiens cDNA clone 309329 5'). The predicted amino acid sequence disclosed herein for ij167\_5 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted ij167\_5 protein demonstrated at least some identity with sequences identified as M96653 (adenylyl cyclase, type 6 [Mus musculus]). Based upon identity, ij167\_5 proteins and each identical protein or peptide may share at least some activity. The TopPredII computer program predicts a potential transmembrane domain within the ij167\_5 protein sequence, centered around amino acid 40 of SEQ ID NO:16.

#### 15        Deposit of Clones

Clones bn97\_1, bn268\_11, cb96\_10, cb213\_11, cj457\_4, cz653\_11, dx138\_4, and ij167\_5 were deposited on September 4, 1997 with the American Type Culture Collection (10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and were given the accession number ATCC 98535, from which each clone comprising a particular polynucleotide is obtainable. All restrictions on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent, except for the requirements specified in 37 C.F.R. § 1.808(b), and the term of the deposit will comply with 37 C.F.R. § 1.806.

Each clone has been transfected into separate bacterial cells (*E. coli*) in this composite deposit. Each clone can be removed from the vector in which it was deposited by performing an EcoRI/NotI digestion (5' site, EcoRI; 3' site, NotI) to produce the appropriate fragment for such clone. Each clone was deposited in either the pED6 or pNOTs vector depicted in Figures 1A and 1B, respectively. The pED6dpc2 vector ("pED6") was derived from pED6dpc1 by insertion of a new polylinker to facilitate cDNA cloning (Kaufman *et al.*, 1991, *Nucleic Acids Res.* 19: 4485-4490); the pNOTs vector was derived from pMT2 (Kaufman *et al.*, 1989, *Mol. Cell. Biol.* 9: 946-958) by deletion of the DHFR sequences, insertion of a new polylinker, and insertion of the M13 origin of replication in the ClaI site. In some instances, the deposited clone can become "flipped"

(i.e., in the reverse orientation) in the deposited isolate. In such instances, the cDNA insert can still be isolated by digestion with EcoRI and NotI. However, NotI will then produce the 5' site and EcoRI will produce the 3' site for placement of the cDNA in proper orientation for expression in a suitable vector. The cDNA may also be expressed from the  
5 vectors in which they were deposited.

Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe or probes should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences  
10 provided herein, or from a combination of those sequences. The sequence of an oligonucleotide probe that was used to isolate or to sequence each full-length clone is identified below, and should be most reliable in isolating the clone of interest.

<u>Clone</u>	<u>Probe Sequence</u>
15 bn97_1	SEQ ID NO:17
bn268_11	SEQ ID NO:18
cb96_10	SEQ ID NO:19
cb213_11	SEQ ID NO:20
cj457_4	SEQ ID NO:21
20 cz653_11	SEQ ID NO:22
dx138_4	SEQ ID NO:23
ij167_5	SEQ ID NO:24

In the sequences listed above which include an N at position 2, that position is occupied  
25 in preferred probes/primers by a biotinylated phosphoramidite residue rather than a nucleotide (such as , for example, that produced by use of biotin phosphoramidite (1-dimethoxytrityloxy-2-(N-biotinyl-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite) (Glen Research, cat. no. 10-1953)).

The design of the oligonucleotide probe should preferably follow these  
30 parameters:

- (a) It should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any;
- (b) It should be designed to have a  $T_m$  of approx. 80 ° C (assuming 2° for each A or T and 4 degrees for each G or C).

The oligonucleotide should preferably be labeled with  $\gamma$ - $^{32}\text{P}$  ATP (specific activity 6000 Ci/mmol) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately  $4 \times 10^6$  dpm/pmol.

The bacterial culture containing the pool of full-length clones should preferably be thawed and 100  $\mu\text{L}$  of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100  $\mu\text{g}/\text{ml}$ . The culture should preferably be grown to saturation at  $37^\circ\text{C}$ , and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100  $\mu\text{g}/\text{ml}$  and agar at 1.5% in a 150 mm petri dish when grown overnight at  $37^\circ\text{C}$ . Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them.

The filter is then preferably incubated at  $65^\circ\text{C}$  for 1 hour with gentle agitation in 6X SSC (20X stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100  $\mu\text{g}/\text{ml}$  of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to  $1 \times 10^6$  dpm/mL. The filter is then preferably incubated at  $65^\circ\text{C}$  with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2X SSC/0.5% SDS at room temperature without agitation, preferably followed by 500 mL of 2X SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1X SSC/0.5% SDS at  $65^\circ\text{C}$  for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

The positive colonies are picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridization analysis, or DNA sequencing.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, *Bio/Technology* 10, 773-778 (1992) and in R.S. McDowell, *et al.*, *J. Amer. Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein of the invention.

The present invention also provides both full-length and mature forms of the disclosed proteins. The full-length form of the such proteins is identified in the sequence listing by translation of the nucleotide sequence of each disclosed clone. The mature form(s) of such protein may be obtained by expression of the disclosed full-length polynucleotide (preferably those deposited with ATCC) in a suitable mammalian cell or other host cell. The sequence(s) of the mature form(s) of the protein may also be determinable from the amino acid sequence of the full-length form.

The present invention also provides genes corresponding to the polynucleotide sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA polynucleotide sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that has been separated from the adjacent coding sequences, if any, present in the genome of the organism from which the gene was isolated.

The chromosomal location corresponding to the polynucleotide sequences disclosed herein may also be determined, for example by hybridizing appropriately

labeled polynucleotides of the present invention to chromosomes *in situ*. It may also be possible to determine the corresponding chromosomal location for a disclosed polynucleotide by identifying significantly similar nucleotide sequences in public databases, such as expressed sequence tags (ESTs), that have already been mapped to  
5 particular chromosomal locations. For at least some of the polynucleotide sequences disclosed herein, public database sequences having at least some similarity to the polynucleotide of the present invention have been listed by database accession number. Searches using the GenBank accession numbers of these public database sequences can then be performed at an Internet site provided by the National Center for Biotechnology  
10 Information having the address <http://www.ncbi.nlm.nih.gov/UniGene/>, in order to identify "UniGene clusters" of overlapping sequences. Many of the "UniGene clusters" so identified will already have been mapped to particular chromosomal sites.

Organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein are provided. The  
15 desired change in gene expression can be achieved through the use of antisense polynucleotides or ribozymes that bind and/or cleave the mRNA transcribed from the gene (Albert and Morris, 1994, *Trends Pharmacol. Sci.* 15(7): 250-254; Lavarosky *et al.*, 1997, *Biochem. Mol. Med.* 62(1): 11-22; and Hampel, 1998, *Prog. Nucleic Acid Res. Mol. Biol.* 58: 1-39; all of which are incorporated by reference herein). Transgenic animals that have  
20 multiple copies of the gene(s) corresponding to the polynucleotide sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. Transgenic animals that have modified genetic control regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are  
25 also provided (see European Patent No. 0 649 464 B1, incorporated by reference herein). In addition, organisms are provided in which the gene(s) corresponding to the polynucleotide sequences disclosed herein have been partially or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) or through deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation  
30 can be accomplished through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, *Bioessays* 14(9): 629-633; Zwaal *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90(16): 7431-7435; Clark *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91(2): 719-722; all of which are incorporated by reference herein), or through homologous recombination, preferably detected by positive/negative genetic selection strategies (Mansour *et al.*, 1988,



*Nature* 336: 348-352; U.S. Patent Nos. 5,464,764; 5,487,992; 5,627,059; 5,631,153; 5,614,396; 5,616,491; and 5,679,523; all of which are incorporated by reference herein). These organisms with altered gene expression are preferably eukaryotes and more preferably are mammals. Such organisms are useful for the development of non-human models for the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the protein product(s) of the corresponding gene(s).

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25% (more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

In particular, sequence identity may be determined using WU-BLAST (Washington University BLAST) version 2.0 software, which builds upon WU-BLAST version 1.4, which in turn is based on the public domain NCBI-BLAST version 1.4 (Altschul and Gish, 1996, Local alignment statistics, Doolittle *ed.*, *Methods in Enzymology* 266: 460-480; Altschul *et al.*, 1990, Basic local alignment search tool, *Journal of Molecular Biology* 215: 403-410; Gish and States, 1993, Identification of protein coding regions by database similarity search, *Nature Genetics* 3: 266-272; Karlin and Altschul, 1993, Applications and statistics for multiple high-scoring segments in molecular sequences, *Proc. Natl. Acad. Sci. USA* 90: 5873-5877; all of which are incorporated by

reference herein). WU-BLAST version 2.0 executable programs for several UNIX platforms can be downloaded from <ftp://blast.wustl.edu/blast/executables>. The complete suite of search programs (BLASTP, BLASTN, BLASTX, TBLASTN, and TBLASTX) is provided at that site, in addition to several support programs. WU-BLAST 2.0 is

5 copyrighted and may not be sold or redistributed in any form or manner without the express written consent of the author; but the posted executables may otherwise be freely used for commercial, nonprofit, or academic purposes. In all search programs in the suite -- BLASTP, BLASTN, BLASTX, TBLASTN and TBLASTX -- the gapped alignment routines are integral to the database search itself, and thus yield much better sensitivity and

10 selectivity while producing the more easily interpreted output. Gapping can optionally be turned off in all of these programs, if desired. The default penalty (Q) for a gap of length one is  $Q=9$  for proteins and BLASTP, and  $Q=10$  for BLASTN, but may be changed to any integer value including zero, one through eight, nine, ten, eleven, twelve through twenty, twenty-one through fifty, fifty-one through one hundred, etc. The default per-residue

15 penalty for extending a gap (R) is  $R=2$  for proteins and BLASTP, and  $R=10$  for BLASTN, but may be changed to any integer value including zero, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve through twenty, twenty-one through fifty, fifty-one through one hundred, etc. Any combination of values for Q and R can be used in order to align sequences so as to maximize overlap and identity while minimizing sequence gaps.

20 The default amino acid comparison matrix is BLOSUM62, but other amino acid comparison matrices such as PAM can be utilized.

Species homologues of the disclosed polynucleotides and proteins are also provided by the present invention. As used herein, a "species homologue" is a protein or polynucleotide with a different species of origin from that of a given protein or

25 polynucleotide, but with significant sequence similarity to the given protein or polynucleotide. Preferably, polynucleotide species homologues have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% identity) with the given polynucleotide, and protein species homologues have at least 30% sequence identity (more preferably, at least 45% identity; most preferably at least 60% identity) with

30 the given protein, where sequence identity is determined by comparing the nucleotide sequences of the polynucleotides or the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Species

homologues may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species. Preferably, species homologues are those isolated from mammalian species. Most preferably, species homologues are those isolated from certain mammalian species such as, for example, *Pan troglodytes*, *Gorilla gorilla*, *Pongo pygmaeus*, *Hylobates concolor*, *Macaca mulatta*, *Papio papio*, *Papio hamadryas*, *Cercopithecus aethiops*, *Cebus capucinus*, *Aotus trivirgatus*, *Sanguinus oedipus*, *Microcebus murinus*, *Mus musculus*, *Rattus norvegicus*, *Cricetulus griseus*, *Felis catus*, *Mustela vison*, *Canis familiaris*, *Oryctolagus cuniculus*, *Bos taurus*, *Ovis aries*, *Sus scrofa*, and *Equus caballus*, for which genetic maps have been created allowing the identification of syntenic relationships between the genomic organization of genes in one species and the genomic organization of the related genes in another species (O'Brien and Seuánez, 1988, *Ann. Rev. Genet.* 22: 323-351; O'Brien *et al.*, 1993, *Nature Genetics* 3:103-112; Johansson *et al.*, 1995, *Genomics* 25: 682-690; Lyons *et al.*, 1997, *Nature Genetics* 15: 47-56; O'Brien *et al.*, 1997, *Trends in Genetics* 13(10): 393-399; Carver and Stubbs, 1997, *Genome Research* 7:1123-1137; all of which are incorporated by reference herein).

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotides which also encode proteins which are identical or have significantly similar sequences to those encoded by the disclosed polynucleotides. Preferably, allelic variants have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% identity) with the given polynucleotide, where sequence identity is determined by comparing the nucleotide sequences of the polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps. Allelic variants may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from individuals of the appropriate species.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

The present invention also includes polynucleotides that hybridize under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as

stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

	Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp) <sup>‡</sup>	Hybridization Temperature and Buffer <sup>†</sup>	Wash Temperature and Buffer <sup>†</sup>
5	A	DNA:DNA	≥ 50	65°C; 1xSSC -or- 42°C; 1xSSC, 50% formamide	65°C; 0.3xSSC
	B	DNA:DNA	<50	T <sub>B</sub> *; 1xSSC	T <sub>B</sub> *; 1xSSC
	C	DNA:RNA	≥ 50	67°C; 1xSSC -or- 45°C; 1xSSC, 50% formamide	67°C; 0.3xSSC
	D	DNA:RNA	<50	T <sub>D</sub> *; 1xSSC	T <sub>D</sub> *; 1xSSC
	E	RNA:RNA	≥ 50	70°C; 1xSSC -or- 50°C; 1xSSC, 50% formamide	70°C; 0.3xSSC
10	F	RNA:RNA	<50	T <sub>F</sub> *; 1xSSC	T <sub>F</sub> *; 1xSSC
	G	DNA:DNA	≥ 50	65°C; 4xSSC -or- 42°C; 4xSSC, 50% formamide	65°C; 1xSSC
	H	DNA:DNA	<50	T <sub>H</sub> *; 4xSSC	T <sub>H</sub> *; 4xSSC
	I	DNA:RNA	≥ 50	67°C; 4xSSC -or- 45°C; 4xSSC, 50% formamide	67°C; 1xSSC
	J	DNA:RNA	<50	T <sub>J</sub> *; 4xSSC	T <sub>J</sub> *; 4xSSC
15	K	RNA:RNA	≥ 50	70°C; 4xSSC -or- 50°C; 4xSSC, 50% formamide	67°C; 1xSSC
	L	RNA:RNA	<50	T <sub>L</sub> *; 2xSSC	T <sub>L</sub> *; 2xSSC
	M	DNA:DNA	≥ 50	50°C; 4xSSC -or- 40°C; 6xSSC, 50% formamide	50°C; 2xSSC
	N	DNA:DNA	<50	T <sub>N</sub> *; 6xSSC	T <sub>N</sub> *; 6xSSC
	O	DNA:RNA	≥ 50	55°C; 4xSSC -or- 42°C; 6xSSC, 50% formamide	55°C; 2xSSC
20	P	DNA:RNA	<50	T <sub>P</sub> *; 6xSSC	T <sub>P</sub> *; 6xSSC
	Q	RNA:RNA	≥ 50	60°C; 4xSSC -or- 45°C; 6xSSC, 50% formamide	60°C; 2xSSC
	R	RNA:RNA	<50	T <sub>R</sub> *; 4xSSC	T <sub>R</sub> *; 4xSSC

<sup>‡</sup> The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

<sup>†</sup> SSPE (1xSSPE is 0.15M NaCl, 10mM NaH<sub>2</sub>PO<sub>4</sub>, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

\*T<sub>B</sub> - T<sub>R</sub>: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T<sub>m</sub>) of the hybrid, where T<sub>m</sub> is determined according to the following equations. For hybrids less than 18 base pairs in length, T<sub>m</sub>(°C) = 2(# of A + T bases) + 4(# of G +

C bases). For hybrids between 18 and 49 base pairs in length,  $T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G+C) - (600/N)$ , where N is the number of bases in the hybrid, and  $[\text{Na}^+]$  is the concentration of sodium ions in the hybridization buffer ( $[\text{Na}^+]$  for 1xSSC = 0.165 M).

- 5 Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and *Current Protocols in Molecular Biology*, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.
- 10 Preferably, each such hybridizing polynucleotide has a length that is at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which it hybridizes,
- 15 where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in

20 Kaufman *et al.*, *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control

25 sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster

30 Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLabs (Beverly, MA), Pharmacia (Piscataway, NJ) and

Invitrogen Corporation (Carlsbad, CA), respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from the Eastman Kodak Company. (New Haven, CT).

5           Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus  
10       purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

          The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the  
15       protein.

          The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational  
20       characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

          The proteins provided herein also include proteins characterized by amino acid  
25       sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For  
30       example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

## **USES AND BIOLOGICAL ACTIVITY**

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

### **Research Uses and Utilities**

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, those described in Gyuris *et al.*, 1993, *Cell* 75: 791-803 and in Rossi *et al.*, 1997, *Proc. Natl. Acad.*



*Sci. USA* 94: 8405-8410, all of which are incorporated by reference herein) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to  
5 determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a  
10 particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can  
15 also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in  
20 the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

#### 25        Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can  
30 be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

### Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors  
5 discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3,  
10 MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those  
15 described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., *J. Immunol.* 137:3494-3500, 1986; Bertagnolli et al., *J. Immunol.* 145:1706-1712, 1990; Bertagnolli et al., *Cellular Immunology*  
20 133:327-341, 1991; Bertagnolli, et al., *J. Immunol.* 149:3778-3783, 1992; Bowman et al., *J. Immunol.* 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a.  
25 Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon  $\gamma$ , Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine  
30 Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., *J. Exp. Med.* 173:1205-1211, 1991; Moreau et al., *Nature* 336:690-692, 1988; Greenberger et al., *Proc. Natl. Acad. Sci. U.S.A.* 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in*

- Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991;
- 5 Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

- Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring
- 10 proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al.,
- 15 Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

#### Immune Stimulating or Suppressing Activity

- 20 A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well
- 25 as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses,
- 30 herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the

molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to  
5 anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

10 The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as  
15 described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

20 Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms.  
25 Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from  
30 the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/*lpr/lpr* mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and

murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy.

- 5 Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B  
10 lymphocyte antigens systemically.

- Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in*  
15 *vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a  
20 costimulatory signal to, and thereby activate, T cells *in vivo*.

- In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present  
25 invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The  
30 transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary

costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I  $\alpha$  chain protein and  $\beta_2$  microglobulin protein or an MHC class II  $\alpha$  chain protein and an MHC class II  $\beta$  chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowman et al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro*

antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., *J. Immunol.* 137:3494-3500, 1986; Takai et al., *J. Immunol.* 140:508-512, 1988; Bertagnolli et al., *J. Immunol.* 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., *J. Immunol.* 134:536-544, 1995; Inaba et al., *Journal of Experimental Medicine* 173:549-559, 1991; Macatonia et al., *Journal of Immunology* 154:5071-5079, 1995; Porgador et al., *Journal of Experimental Medicine* 182:255-260, 1995; Nair et al., *Journal of Virology* 67:4062-4069, 1993; Huang et al., *Science* 264:961-965, 1994; Macatonia et al., *Journal of Experimental Medicine* 169:1255-1264, 1989; Bhardwaj et al., *Journal of Clinical Investigation* 94:797-807, 1994; and Inaba et al., *Journal of Experimental Medicine* 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., *Cytometry* 13:795-808, 1992; Gorczyca et al., *Leukemia* 7:659-670, 1993; Gorczyca et al., *Cancer Research* 53:1945-1951, 1993; Itoh et al., *Cell* 66:233-243, 1991; Zacharchuk, *Journal of Immunology* 145:4037-4045, 1990; Zamai et al., *Cytometry* 14:891-897, 1993; Gorczyca et al., *International Journal of Oncology* 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., *Blood* 84:111-117, 1994; Fine et al., *Cellular Immunology* 155:111-122, 1994; Galy et al., *Blood* 85:2770-2778, 1995; Toki et al., *Proc. Nat. Acad Sci. USA* 88:7548-7551, 1991.

#### Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell



lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. *Cellular Biology* 15:141-151, 1995; Keller et al., *Molecular and Cellular Biology* 13:473-486, 1993; McClanahan et al., *Blood* 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., *Proc. Natl. Acad. Sci. USA* 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and

- Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., *Experimental Hematology* 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long
- 5 term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

10

#### Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns,

15 incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as

20 well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal

25 disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue

30 destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in

circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells  
5 comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues,  
10 and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured  
15 by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium ).

20 Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

#### 25 Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present  
30 invention, alone or in heterodimers with a member of the inhibin  $\alpha$  family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-

$\beta$  group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the  
5 lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et  
10 al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

#### Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity  
15 (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in  
20 treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell  
25 population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured  
30 by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion

- include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

#### Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity.

- 10 As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting  
15 therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

- Assay for hemostatic and thrombolytic activity include, without limitation, those  
20 described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

#### Receptor/Ligand Activity

- 25 A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation,  
30 cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without

limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

- 5        Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987;
- 10    Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

#### Anti-Inflammatory Activity

- 15        Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or
- 20    suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin
- 25    lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

#### Cadherin/Tumor Invasion Suppressor Activity

30        Cadherins are calcium-dependent adhesion molecules that appear to play major roles during development, particularly in defining specific cell types. Loss or alteration of normal cadherin expression can lead to changes in cell adhesion properties linked to tumor growth and metastasis. Cadherin malfunction is also implicated in other human

diseases, such as pemphigus vulgaris and pemphigus foliaceus (auto-immune blistering skin diseases), Crohn's disease, and some developmental abnormalities.

The cadherin superfamily includes well over forty members, each with a distinct pattern of expression. All members of the superfamily have in common conserved  
5 extracellular repeats (cadherin domains), but structural differences are found in other parts of the molecule. The cadherin domains bind calcium to form their tertiary structure and thus calcium is required to mediate their adhesion. Only a few amino acids in the first cadherin domain provide the basis for homophilic adhesion; modification of this recognition site can change the specificity of a cadherin so that instead of recognizing only  
10 itself, the mutant molecule can now also bind to a different cadherin. In addition, some cadherins engage in heterophilic adhesion with other cadherins.

E-cadherin, one member of the cadherin superfamily, is expressed in epithelial cell types. Pathologically, if E-cadherin expression is lost in a tumor, the malignant cells become invasive and the cancer metastasizes. Transfection of cancer cell lines with  
15 polynucleotides expressing E-cadherin has reversed cancer-associated changes by returning altered cell shapes to normal, restoring cells' adhesiveness to each other and to their substrate, decreasing the cell growth rate, and drastically reducing anchorage-independent cell growth. Thus, reintroducing E-cadherin expression reverts carcinomas to a less advanced stage. It is likely that other cadherins have the same invasion  
20 suppressor role in carcinomas derived from other tissue types. Therefore, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be used to treat cancer. Introducing such proteins or polynucleotides into cancer cells can reduce or eliminate the cancerous changes observed in these cells by providing normal cadherin expression.

25 Cancer cells have also been shown to express cadherins of a different tissue type than their origin, thus allowing these cells to invade and metastasize in a different tissue in the body. Proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be substituted in these cells for the inappropriately expressed cadherins, restoring normal cell adhesive properties and  
30 reducing or eliminating the tendency of the cells to metastasize.

Additionally, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be used to generate antibodies recognizing and binding to cadherins. Such antibodies can be used to block the adhesion of inappropriately expressed tumor-cell cadherins, preventing the cells from



forming a tumor elsewhere. Such an anti-cadherin antibody can also be used as a marker for the grade, pathological type, and prognosis of a cancer, i.e. the more progressed the cancer, the less cadherin expression there will be, and this decrease in cadherin expression can be detected by the use of a cadherin-binding antibody.

- 5           Fragments of proteins of the present invention with cadherin activity, preferably a polypeptide comprising a decapeptide of the cadherin recognition site, and polynucleotides of the present invention encoding such protein fragments, can also be used to block cadherin function by binding to cadherins and preventing them from binding in ways that produce undesirable effects. Additionally, fragments of proteins of the present
- 10   invention with cadherin activity, preferably truncated soluble cadherin fragments which have been found to be stable in the circulation of cancer patients, and polynucleotides encoding such protein fragments, can be used to disturb proper cell-cell adhesion.

- Assays for cadherin adhesive and invasive suppressor activity include, without limitation, those described in: Hortsch et al. J Biol Chem 270 (32): 18809-18817, 1995;
- 15   Miyaki et al. Oncogene 11: 2547-2552, 1995; Ozawa et al. Cell 63: 1033-1038, 1990.

#### Tumor Inhibition Activity

- In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities.
- 20   A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating
- 25   or inhibiting factors, agents or cell types which promote tumor growth.

#### Other Activities

- A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious
- 30   agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or cardiac cycles or rhythms;

effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

15

#### **ADMINISTRATION AND DOSING**

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier.

Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects

of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical  
5 compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B  
10 lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be  
15 supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

20 The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation,  
25 monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

30 As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active

ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a  
5 therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines,  
10 lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with  
15 cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or  
20 cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical  
25 composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils  
30 may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein

of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 µg to about 100 mg (preferably about 0.1mg to about 10 mg, more preferably about 0.1 µg to about 1 mg) of protein of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such

antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in

5 R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where

10 abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage,

15 tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or

20 tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the

25 composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

30 The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and

polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other  
5 ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

10       Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

15       A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic  
20 acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells  
25 are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor  
30 (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.



What is claimed is:

1. An isolated polynucleotide selected from the group consisting of:
  - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;
  - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 69 to nucleotide 908;
  - (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 270 to nucleotide 908;
  - (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone bn97\_1 deposited under accession number ATCC 98535;
  - (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone bn97\_1 deposited under accession number ATCC 98535;
  - (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone bn97\_1 deposited under accession number ATCC 98535;
  - (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone bn97\_1 deposited under accession number ATCC 98535;
  - (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;
  - (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:2;
  - (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
  - (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and
  - (l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).
2. The polynucleotide of claim 1 wherein said polynucleotide is operably linked to at least one expression control sequence.
3. A host cell transformed with the polynucleotide of claim 2.

4. The host cell of claim 3, wherein said cell is a mammalian cell.
5. A process for producing a protein encoded by the polynucleotide of claim 2, which process comprises:
  - (a) growing a culture of the host cell of claim 3 in a suitable culture medium; and
  - (b) purifying said protein from the culture.
6. A protein produced according to the process of claim 5.
7. A protein comprising an amino acid sequence selected from the group consisting of:
  - (a) the amino acid sequence of SEQ ID NO:2;
  - (b) the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 83;
  - (c) fragments of the amino acid sequence of SEQ ID NO:2 comprising eight consecutive amino acids of SEQ ID NO:2; and
  - (d) the amino acid sequence encoded by the cDNA insert of clone bn97\_1 deposited under accession number ATCC 98535;the protein being substantially free from other mammalian proteins.
8. The protein of claim 7, wherein said protein comprises the amino acid sequence of SEQ ID NO:2.
9. The protein of claim 7, wherein said protein comprises the amino acid sequence of SEQ ID NO:2 from amino acid 1 to amino acid 83.
10. A composition comprising the protein of claim 7 and a pharmaceutically acceptable carrier.
11. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:1.
12. An isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3;
  - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 562 to nucleotide 777;
  - (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 236 to nucleotide 673;
  - (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone bn268\_11 deposited under accession number ATCC 98535;
  - (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone bn268\_11 deposited under accession number ATCC 98535;
  - (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone bn268\_11 deposited under accession number ATCC 98535;
  - (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone bn268\_11 deposited under accession number ATCC 98535;
  - (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;
  - (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:4;
  - (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
  - (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and
  - (l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).
13. A protein comprising an amino acid sequence selected from the group consisting of:
- (a) the amino acid sequence of SEQ ID NO:4;
  - (b) the amino acid sequence of SEQ ID NO:4 from amino acid 1 to amino acid 37;

(c) fragments of the amino acid sequence of SEQ ID NO:4 comprising eight consecutive amino acids of SEQ ID NO:4; and

(d) the amino acid sequence encoded by the cDNA insert of clone bn268\_11 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins.

14. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:3.

15. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 286 to nucleotide 1686;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 544 to nucleotide 1686;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 365 to nucleotide 1160;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone cb96\_10 deposited under accession number ATCC 98535;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone cb96\_10 deposited under accession number ATCC 98535;

(g) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone cb96\_10 deposited under accession number ATCC 98535;

(h) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone cb96\_10 deposited under accession number ATCC 98535;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:6;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(j).

16. A protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:6;

(b) the amino acid sequence of SEQ ID NO:6 from amino acid 28 to amino acid 395;

(c) fragments of the amino acid sequence of SEQ ID NO:6 comprising eight consecutive amino acids of SEQ ID NO:6; and

(d) the amino acid sequence encoded by the cDNA insert of clone cb96\_10 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins.

17. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:5.

18. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 99 to nucleotide 1049;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 222 to nucleotide 1049;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 632 to nucleotide 998;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone cb213\_11 deposited under accession number ATCC 98535;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone cb213\_11 deposited under accession number ATCC 98535;

(g) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone cb213\_11 deposited under accession number ATCC 98535;

(h) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone cb213\_11 deposited under accession number ATCC 98535;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:8;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and

(m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(j).

19. A protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:8;

(b) the amino acid sequence of SEQ ID NO:8 from amino acid 187 to amino acid 300;

(c) fragments of the amino acid sequence of SEQ ID NO:8 comprising eight consecutive amino acids of SEQ ID NO:8; and

(d) the amino acid sequence encoded by the cDNA insert of clone cb213\_11 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins.

20. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:7.

21. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9;

- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 3003 to nucleotide 3137;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 3072 to nucleotide 3137;
- (d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2713 to nucleotide 3114;
- (e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone cj457\_4 deposited under accession number ATCC 98535;
- (f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone cj457\_4 deposited under accession number ATCC 98535;
- (g) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone cj457\_4 deposited under accession number ATCC 98535;
- (h) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone cj457\_4 deposited under accession number ATCC 98535;
- (i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;
- (j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:10;
- (k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;
- (l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ; and
- (m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(j).

22. A protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10;
- (b) the amino acid sequence of SEQ ID NO:10 from amino acid 1 to amino acid 37;

- (c) fragments of the amino acid sequence of SEQ ID NO:10 comprising eight consecutive amino acids of SEQ ID NO:10; and
  - (d) the amino acid sequence encoded by the cDNA insert of clone cj457\_4 deposited under accession number ATCC 98535;
- the protein being substantially free from other mammalian proteins.

23. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:9.

24. An isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 284 to nucleotide 1357;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 603 to nucleotide 1233;
- (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone cz653\_11 deposited under accession number ATCC 98535;
- (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone cz653\_11 deposited under accession number ATCC 98535;
- (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone cz653\_11 deposited under accession number ATCC 98535;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone cz653\_11 deposited under accession number ATCC 98535;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:12;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and



(l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).

25. A protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:12;
- (b) the amino acid sequence of SEQ ID NO:12 from amino acid 147 to amino acid 358;
- (c) fragments of the amino acid sequence of SEQ ID NO:12 comprising eight consecutive amino acids of SEQ ID NO:12; and
- (d) the amino acid sequence encoded by the cDNA insert of clone cz653\_11 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins.

26. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:11.

27. An isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 621 to nucleotide 1763;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 1461 to nucleotide 1763;
- (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone dx138\_4 deposited under accession number ATCC 98535;
- (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone dx138\_4 deposited under accession number ATCC 98535;
- (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone dx138\_4 deposited under accession number ATCC 98535;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone dx138\_4 deposited under accession number ATCC 98535;

- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:14;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:14;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and
- (l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).

28. A protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:14;
- (b) the amino acid sequence of SEQ ID NO:14 from amino acid 83 to amino acid 229;
- (c) fragments of the amino acid sequence of SEQ ID NO:14 comprising eight consecutive amino acids of SEQ ID NO:14; and
- (d) the amino acid sequence encoded by the cDNA insert of clone dx138\_4 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins.

29. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:13.

30. An isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from nucleotide 119 to nucleotide 295;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 from nucleotide 191 to nucleotide 295;

- (d) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone ij167\_5 deposited under accession number ATCC 98535;
- (e) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone ij167\_5 deposited under accession number ATCC 98535;
- (f) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone ij167\_5 deposited under accession number ATCC 98535;
- (g) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone ij167\_5 deposited under accession number ATCC 98535;
- (h) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:16;
- (i) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:16 having biological activity, the fragment comprising eight consecutive amino acids of SEQ ID NO:16;
- (j) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(g) above;
- (k) a polynucleotide which encodes a species homologue of the protein of (h) or (i) above ; and
- (l) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(i).

31. A protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:16;
- (b) the amino acid sequence of SEQ ID NO:16 from amino acid 1 to amino acid 26;
- (c) fragments of the amino acid sequence of SEQ ID NO:16 comprising eight consecutive amino acids of SEQ ID NO:16; and
- (d) the amino acid sequence encoded by the cDNA insert of clone ij167\_5 deposited under accession number ATCC 98535;

the protein being substantially free from other mammalian proteins.

32. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:15.

FIGURE 1A

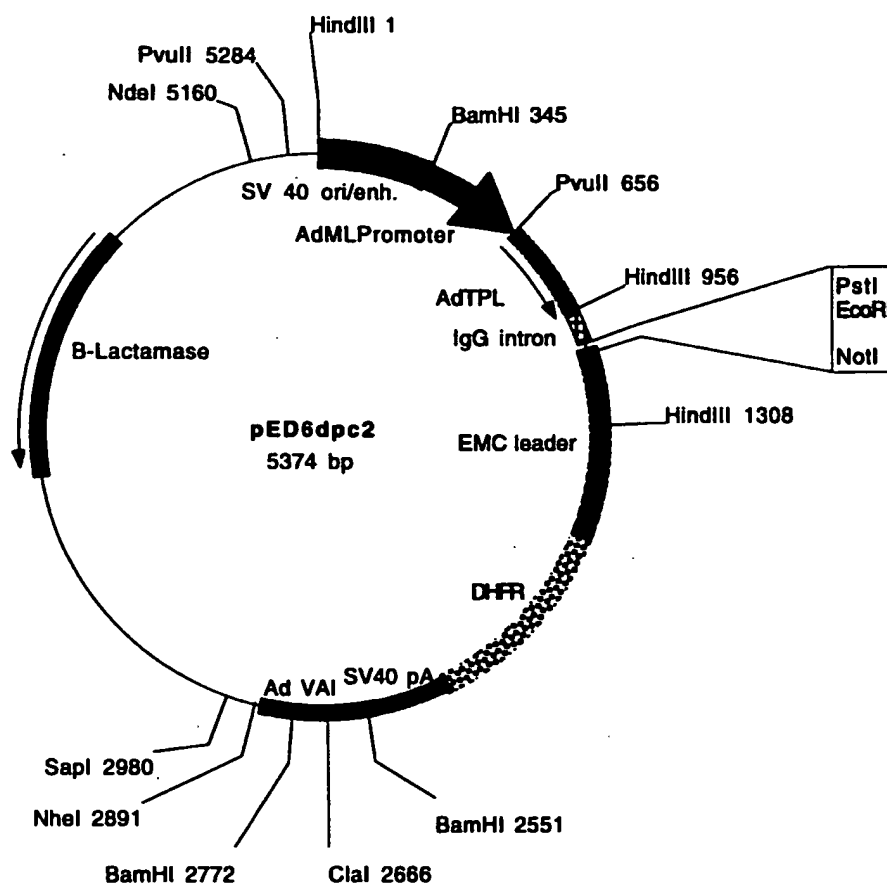
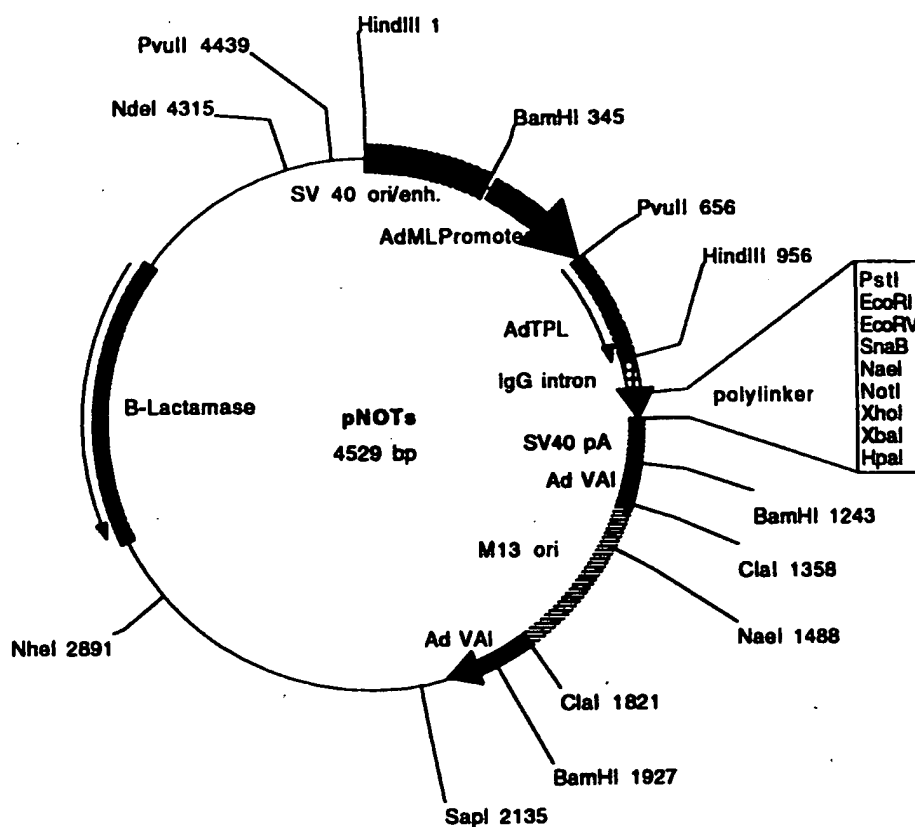


FIGURE 1B



## SEQUENCE LISTING

<110> Jacobs, Kenneth  
McCoy, John M.  
LaVallie, Edward R.  
Racie, Lisa A.  
Evans, Cheryl  
Merberg, David  
Treacy, Maurice  
Agostino, Michael J.  
Spaulding, Vikki  
Genetics Institute, Inc.

**<120> SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM**

<130> 6052A

**<140>**

<141>

<160> 24

&lt;170&gt; PatentIn Ver. 2.0

<210> 1

<211> 1776

**<212> DNA**

<213> Homo sapiens

**<400> 1**

agctcacagt	agcccggcgg	cccagggccaa	tccgaccaca	tttcaactctc	accgctgtag	60
gaatccagat	gcaggccaag	tacagcagca	cgagggaacat	gctggatgat	gatggggaca	120
ccaccatgag	cctgcattct	caagcctctg	ccacaactcg	gcattccagag	ccccggcgca	180
cacagcacag	ggctccctct	tcaacgtggc	gaccagtggc	cctgacctcg	ctgactttgt	240
gcttgggtgt	ctgtataggg	ctggcagccc	tggggctttt	gttttttcag	tactaccagc	300
tctccaatac	tgttcaagac	accattttct	aaatggaaga	aagattagga	aatacgtccc	360
aagagttgca	atctcttcaa	gtccagaata	taaagcttgc	aggaagtctg	cagcatgtgg	420
ctgaaaaaact	ctgtcgtgag	ctgtataaca	aagctggagc	acacaggtgc	agcccttgta	480
cagaacaatg	gaaatggcat	ggagacaatt	gctaccagtt	ctataaagac	agcaaaaagt	540
gggaggactg	taaattatct	tgccttagtg	aaaactctac	catgctgaag	ataaacaaca	600
aagaagacct	ggaatttgcc	gcgtctcaga	ctactctga	gttttctac	tcttattgga	660
cagggtcttt	gcgccctgac	agtggcaagg	cctggctgtg	gatggatgga	acccctttca	720
cttctgaact	gttccatatt	ataatagatg	tcaccagccc	aagaagcaga	gactgtgtgg	780
ccatccttaa	tgggatgatc	ttctcaagg	actgcaaaga	atgaagcgt	tgtgtctgtg	840
agagaagggc	aggaatgggt	aagccagaga	gcctccatgt	ccccctgaa	acattaggcg	900
aagggtgact	attcgccttc	tgcacactaga	aatagcagag	tgagccagtc	ggtgccaaag	960
caagggtctg	ttgagacatt	gggaatatga	acataactcag	gaagaactct	ctctctgact	1020
agtacaaaat	gggtttctct	gtttctctgt	caggatcacc	agcattttctg	agcttgggtt	1080
tatgcacgta	tttaacagtc	acaagaagtc	ttattttacat	gccaccaacc	aacctcagaa	1140
accataatg	tcattctgct	tcttggctta	gagataactt	ttagctctct	ttcttctcaa	1200
tgtctaatat	cacctccctg	ttttcatgtc	ttcctttcac	ttggtggaat	aagaaacttt	1260
ttgaagtaga	ggaaatacat	tgaggttaaca	tcctttttct	tgacagtcaa	gtatgccatt	1320
agaaattggc	agtcacttcc	cagattgtac	cagcaaatac	acaaggaaat	ctttttgttt	1380
gtttcagttc	atactagtct	cttcccaatc	catcagtaaa	gaccccatct	gccttgtcca	1440
tgccgtttcc	caacagggat	gtcacttgat	atgagaatct	caaattctcaa	tgctttataa	1500
gcattccttc	ctgtgtccat	taagactctg	ataattgtct	ccccctcata	ggaattttctc	1560
ccaggaaaaga	aatatatccc	catctccgtt	tcatatcaga	actaccgtcc	ccgatattcc	1620
cttcagagag	attaaagacc	agaaaaaatg	gagcctcttc	atctgcacct	gtaatatgtt	1680
cagttccctat	tttcttccat	tgaccataat	ttataccttt	caggtactga	agatttaata	1740
ataataaatg	taaatactgt	gaaaaaaaaa	aaaaaa			1776

&lt;210&gt; 2

&lt;211&gt; 280

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 2

```

Met Gln Ala Lys Tyr Ser Ser Thr Arg Asp Met Leu Asp Asp Asp Gly
 1              5              10              15

Asp Thr Thr Met Ser Leu His Ser Gln Ala Ser Ala Thr Thr Arg His
      20              25              30

Pro Glu Pro Arg Arg Thr Glu His Arg Ala Pro Ser Ser Thr Trp Arg
      35              40              45

Pro Val Ala Leu Thr Leu Leu Thr Leu Cys Leu Val Leu Leu Ile Gly
      50              55              60

Leu Ala Ala Leu Gly Leu Leu Phe Phe Gln Tyr Tyr Gln Leu Ser Asn
      65              70              75              80

Thr Gly Gln Asp Thr Ile Ser Gln Met Glu Glu Arg Leu Gly Asn Thr
      85              90              95

Ser Gln Glu Leu Gln Ser Leu Gln Val Gln Asn Ile Lys Leu Ala Gly
      100             105             110

Ser Leu Gln His Val Ala Glu Lys Leu Cys Arg Glu Leu Tyr Asn Lys
      115             120             125

Ala Gly Ala His Arg Cys Ser Pro Cys Thr Glu Gln Trp Lys Trp His
      130             135             140

Gly Asp Asn Cys Tyr Gln Phe Tyr Lys Asp Ser Lys Ser Trp Glu Asp
      145             150             155             160

Cys Lys Tyr Phe Cys Leu Ser Glu Asn Ser Thr Met Leu Lys Ile Asn
      165             170             175

Lys Gln Glu Asp Leu Glu Phe Ala Ala Ser Gln Ser Tyr Ser Glu Phe
      180             185             190

Phe Tyr Ser Tyr Trp Thr Gly Leu Leu Arg Pro Asp Ser Gly Lys Ala
      195             200             205

Trp Leu Trp Met Asp Gly Thr Pro Phe Thr Ser Glu Leu Phe His Ile
      210             215             220

Ile Ile Asp Val Thr Ser Pro Arg Ser Arg Asp Cys Val Ala Ile Leu
      225             230             235             240

Asn Gly Met Ile Phe Ser Lys Asp Cys Lys Glu Leu Lys Arg Cys Val
      245             250             255

Cys Glu Arg Arg Ala Gly Met Val Lys Pro Glu Ser Leu His Val Pro
      260             265             270

Pro Glu Thr Leu Gly Glu Gly Asp
      275             280

```

<210> 3  
 <211> 947  
 <212> DNA  
 <213> Homo sapiens

<400> 3  
 caactatccc ataatttatt tattcttctt caatgtttgt aaagtgcag agtcatgttc 60  
 acacttgaag tctagtagtg cactgtaata attcattttt taaaagatta tttaatgccc 120  
 atttcaaaat acagtagttt acacagctac agaaacaatt tggggcaagt tttaaaacac 180  
 tgaaacagta atagttattg gtgtcacata aaactgattt gttttttaca gccaaacctc 240  
 tgtcagtcag aggcatcat tagttttata catgtaattt gaaaatcact aaacctcggt 300  
 ttctcagcag caataattta agaggcttca aaaatataat ttcactctta tttagttatt 360  
 ttctctgggg ggatttttac gtaatttttt tatgaaaaga caaatgcag ttgagataac 420  
 ttctggggatt aaaatagtct ttgtctttac ttttttggtt tcctaaaaca actttattga 480  
 ctttttagtcc atactgttat atttttgtct taaagaaaat ttaaaactaca aataccaaaa 540  
 gaaaacattt taaatttagg gatgagactt tgggtgtatc tgggtctagg tttaatgaac 600  
 acatctgggg ttaagtggc atttcttcac atctccacac ccacaccaac catcacagcc 660  
 ccccaaccaac cttctcccaa ccccaaaaagc attgtccagg gatatagatt ttaccaaaagg 720  
 cttctctggga agacgaggga gcaacacttt agattaaatg tgatcagact ttcctattag 780  
 atatggctct tctgtctctt gttatccccc tgacagctct gccataaagt cccttctcct 840  
 catccttccc aaacaggctg tataagtgtt ttgaggtaat taaactcttt cctccagttt 900  
 acaaatatca cttaacaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa 947

<210> 4  
 <211> 72  
 <212> PRT  
 <213> Homo sapiens

<400> 4  
 Met Arg Leu Trp Cys Ile Val Gly Leu Gly Leu Met Asn Thr Ser Gly  
 1 5 10 15  
 Val Lys Leu Ala Phe Leu His Ile Ser Thr Pro Thr Pro Thr Ile Thr  
 20 25 30  
 Ala Pro His Gln Pro Ser Pro Asn Pro Lys Ser Ile Val Gln Gly Tyr  
 35 40 45  
 Arg Phe Tyr Gln Arg Leu Pro Gly Lys Thr Arg Glu Gln His Phe Arg  
 50 55 60  
 Leu Asn Val Ile Arg Leu Ser Tyr  
 65 70

<210> 5  
 <211> 2120  
 <212> DNA  
 <213> Homo sapiens

<400> 5  
 cgctgacttg ggcaatgggg ccggtggggg ttgggggcgg aagagaccct cgggggttgag 60  
 aagtatgttg tggcctttcg tcccctgtaa aacattgtca cacggtgttg ggcggcagcg 120  
 ctggatcttt gcaaggctat ttgggcattc tgctggatat atgttcgtaa ataccaaagt 180  
 cgggcgggaaa gtgaagtgtg ctccaccata acagcaattt tttctctagc aattgcactt 240  
 atcacatcag cacttctacc agtggatata tttttggttt cttacatgaa aaatcaaaat 300  
 ggtacattta aggactgggc taatgctaag gtcagcagac agattgagga cactgtatta 360  
 tacggttact atactttata ttctgttata ttgttctgtg tgttctctg gatccctttt 420  
 gtctacttct attatgaaga aaaggatgat gatgatacta gtaaattgtac tcaaattaaa 480



```

acggcactca agtatacttt gggatttggt gtgatttggt cactgcttct tttagttggt 540
gcctttgttc cattgaatgt tcccaataac aaaaattcta cagagtggga aaaagtgaag 600
tccctatttg aagaacttgg aagtagtcat ggtttagctg cattgtcatt ttctatcagt 660
tctctgacct tgattggaat gttggcagct ataacttaca cagcctatgg catgtctgcg 720
ttacctttaa atctgataaa aggcactaga agcgctgctt atgaacgttt ggaaaacact 780
gaagacattg aagaagtaga acaacacatt caaacgatta aatcaaaaag caaagatggt 840
cgacctttgc cagcaaggga taaacgcgcc ttaaaacaat ttgaagaaag gttacgaaca 900
cttaagaaga gagagaggca tttagaattc attgaaaaca gctggtggac aaaattttgt 960
ggcgctctgc gtccccgtaa gatcgtctgg ggaatatttt tcatcttagt tgcatgtctg 1020
tttgtaattt ctcttttctt gtcaaattta gataaagctc ttcattcagc tggaatagat 1080
tctggtttca taatttttgg agctaacctg agtaatccac tgaatatgct tttgccttta 1140
ctacaaacag ttttccctct tgattatatt ctataacaa ttattattat gtactttatt 1200
tttacttcaa tggcaggaat tcgaaatatt ggcatatggt tcttttggat tagattatat 1260
aaaatcagaa gaggtagaac caggcccaa gcactccttt ttctctgcat gatacttctg 1320
cttattgtcc ttcacactag ctacatgatt tatagtcttg ctccccaata tgttatgtat 1380
ggaagccaaa attacttaat agagactaat ataacttctg ataatacata aggcaattca 1440
accctttctg tgccaaagag atgtgatgca gatgctcctg aagatcagtg tactgttacc 1500
cggacatacc tattccttca caagttcttg ttcttcagtg ctgcttacta ttttggtaac 1560
tgggcctttc ttggggattt tttgattgga ttaattgtat cctgttgtaa aggggaagaaa 1620
tcggttattg aaggagtaga tgaagattca gacataagtg atgatgagcc ctctgtctat 1680
tctgcttgac agccttctgt cttaaagggt ttataatgct gactgaatat ctgttatgca 1740
tttttaagat attaaactaa cattaggatt tgctaactag ctttcatcaa aaatgggagc 1800
atggctataa gacaactata ttttattata tgttttctga agtaacattg tatcatagat 1860
taacatttta aattaccata atcatgctat gtaaataata gactactggc tttgtgaggg 1920
aatgtttgtg caaaattttt tcctctaatt tataatagtg ttaaattgat taaaaatctt 1980
ccagaattaa tattcccttt tgtcactttt tgaaaacata ataaatcatt tgtatctgtg 2040
ccttaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 2100
aaaaaaaaa aaaaaaaaaa

```

&lt;210&gt; 6

&lt;211&gt; 467

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 6

```

Met Lys Asn Gln Asn Gly Thr Phe Lys Asp Trp Ala Asn Ala Asn Val
  1                      5                      10                      15

```

```

Ser Arg Gln Ile Glu Asp Thr Val Leu Tyr Gly Tyr Tyr Thr Leu Tyr
      20                      25                      30

```

```

Ser Val Ile Leu Phe Cys Val Phe Phe Trp Ile Pro Phe Val Tyr Phe
      35                      40                      45

```

```

Tyr Tyr Glu Glu Lys Asp Asp Asp Asp Thr Ser Lys Cys Thr Gln Ile
      50                      55                      60

```

```

Lys Thr Ala Leu Lys Tyr Thr Leu Gly Phe Val Val Ile Cys Ala Leu
      65                      70                      75                      80

```

```

Leu Leu Leu Val Gly Ala Phe Val Pro Leu Asn Val Pro Asn Asn Lys
      85                      90                      95

```

```

Asn Ser Thr Glu Trp Glu Lys Val Lys Ser Leu Phe Glu Glu Leu Gly
      100                      105                      110

```

```

Ser Ser His Gly Leu Ala Ala Leu Ser Phe Ser Ile Ser Ser Leu Thr
      115                      120                      125

```

```

Leu Ile Gly Met Leu Ala Ala Ile Thr Tyr Thr Ala Tyr Gly Met Ser

```

130	135	140
Ala Leu Pro Leu Asn Leu Ile Lys Gly Thr Arg Ser Ala Ala Tyr Glu		
145	150	155 160
Arg Leu Glu Asn Thr Glu Asp Ile Glu Glu Val Glu Gln His Ile Gln		
	165	170 175
Thr Ile Lys Ser Lys Ser Lys Asp Gly Arg Pro Leu Pro Ala Arg Asp		
	180	185 190
Lys Arg Ala Leu Lys Gln Phe Glu Glu Arg Leu Arg Thr Leu Lys Lys		
	195	200 205
Arg Glu Arg His Leu Glu Phe Ile Glu Asn Ser Trp Trp Thr Lys Phe		
	210	215 220
Cys Gly Ala Leu Arg Pro Leu Lys Ile Val Trp Gly Ile Phe Phe Ile		
	225	230 235 240
Leu Val Ala Leu Leu Phe Val Ile Ser Leu Phe Leu Ser Asn Leu Asp		
	245	250 255
Lys Ala Leu His Ser Ala Gly Ile Asp Ser Gly Phe Ile Ile Phe Gly		
	260	265 270
Ala Asn Leu Ser Asn Pro Leu Asn Met Leu Leu Pro Leu Leu Gln Thr		
	275	280 285
Val Phe Pro Leu Asp Tyr Ile Leu Ile Thr Ile Ile Ile Met Tyr Phe		
	290	295 300
Ile Phe Thr Ser Met Ala Gly Ile Arg Asn Ile Gly Ile Trp Phe Phe		
	305	310 315 320
Trp Ile Arg Leu Tyr Lys Ile Arg Arg Gly Arg Thr Arg Pro Gln Ala		
	325	330 335
Leu Leu Phe Leu Cys Met Ile Leu Leu Leu Ile Val Leu His Thr Ser		
	340	345 350
Tyr Met Ile Tyr Ser Leu Ala Pro Gln Tyr Val Met Tyr Gly Ser Gln		
	355	360 365
Asn Tyr Leu Ile Glu Thr Asn Ile Thr Ser Asp Asn His Lys Gly Asn		
	370	375 380
Ser Thr Leu Ser Val Pro Lys Arg Cys Asp Ala Asp Ala Pro Glu Asp		
	385	390 395 400
Gln Cys Thr Val Thr Arg Thr Tyr Leu Phe Leu His Lys Phe Trp Phe		
	405	410 415
Phe Ser Ala Ala Tyr Tyr Phe Gly Asn Trp Ala Phe Leu Gly Val Phe		
	420	425 430
Leu Ile Gly Leu Ile Val Ser Cys Cys Lys Gly Lys Lys Ser Val Ile		
	435	440 445
Glu Gly Val Asp Glu Asp Ser Asp Ile Ser Asp Asp Glu Pro Ser Val		

450

455

460

Tyr Ser Ala  
465

&lt;210&gt; 7

&lt;211&gt; 2487

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 7

```

gttccgaaat aaaagatttt gcaaaccact ttcctacgta cgtccactgt agtttttgca 60
gatacaacac tagctgtagc aaagcctatg taaatcatat gatgagcttt catagtaacc 120
gtccaagcaa aagggtttgt atttttaaga agcatttcaga aaatctccgg ggcattactc 180
tagtgtgcct taattgtgat ttcctaagtg atgtttctgg cttagataat atggctacac 240
acttaagtca acataaaact cataacttgc aagttgtaat gcagaaagtt tctgtttgta 300
tcccaacttc tgagcacctt tctgaattaa aaaaagaagc tcccgcaaag gaacaagaac 360
ctgtgtctaa ggaattgca agacctaaac tggctgaaag agaacagaa acatcaaatt 420
ctgaaagtaa acaagataaa gctgcttctt caaaagaaaa aaatggatgt aatgcaaatt 480
catttgaagg ctcatcaaca acaaaaagtg aagaaagcat aacagtttca gataaggaaa 540
atgaaacctg tcttcagac caggaaactg gctcaaaaaa catcgtcagt tgtgattcaa 600
atattggtgc agataaagtg gaaaagaaaa aacaaataca acacgtttgt caggaaatgg 660
agttgaagat gtgccaagt tcagaaaaca taatcttatc tgatcagatt aaagatcaca 720
actccagtga agccagattt tcttcaaaga atattaagga tttgcgatta gcatcagata 780
atgtaagcat tgatcagttt ttgagaaaaa gacatgaacc tgaatctgtt agttctgatg 840
ttagcgagca aggcagtatt catttggaac ctctgactcc atccgaggta cttgagtatg 900
aagccacaga gattcttcag aaaggtagtg gtgaccttc agccaagact gatgaagtag 960
tgtctgatca aacagatgac attcctggag gaaataaccc tagcacaaca gaggcaacag 1020
tagacctgga agatgaaaaa gaaagaagtt gaaattagtc attttaagtt tcagtgtacc 1080
aacgataagg gcatttggaa cagtgcctatc aggtgagctc agtggtgctg ttgtagggtc 1140
agaaatggaa atatgtaagg gaggtcacac atacacttta cctgtatgtt caacctatgt 1200
tatcaaacaa atcaattcac caataatagc atgattagta gggattccca aaaagttttt 1260
aaaaacacga acaggatttt aatgataatt aaatttgcag tggaaaggtc tcatttaatg 1320
gttttcaagg aaatgggatt tgggtgctga catgaattga tgatattagt aatatttata 1380
aagcctttca aactccatc aatcctaagc taaaaatctt tattacctgt atatcctttt 1440
cagttaactg agaggaaggg atttggaac catgtacttt tggggagtaa ttgattaaaa 1500
acaatggctg atggcattg ttaatgaagg ctttatttgt gaggatgatg ctggtaaatg 1560
gagcatgctt agagtactaa attgatctaa tgagaatttg gatgaacata aacttaattt 1620
tggatttaat ataaccattc agtcagacgc atgtaaacag aatatttgaa tctttgtacc 1680
tccatacaag tgttagcctg ccaggctgta agcttacctt aattaaactt tcagtgaag 1740
tggaattatt aagatataaa tttataattg tgctttttgt cagtgtgtaa gctgtgtaga 1800
aattccttga tgtattagtt gtattaatgt aaagtagaaa ccattgttg aaactcctgt 1860
agctattatg cttttaatat tgttttaatt atcttcctta gaaataggcc cataaaaatg 1920
gtctggaagc caaaccaaag tatggtataa tgtagatatt gtaaagcagt aaactgaaaa 1980
catgtcctgg catgtattca gccatgttta agtgactttt ctgtaattgt aaaataaaaa 2040
cttcaaatgg gacctaatac agtgatgtaa aagaactggg tttggaaatt tagcctaatt 2100
tatctataag atggctgcta aattgatttt tcagttcttt ttatcatcta gaataataa 2160
gatatagaaa tgaataatat gaagaacagt agtttgcttt gaaatactaa taaactttta 2220
tttaaaatgc ttcattttta cttcttaaaa tgtgcttttg attcttaaat tttgtttcac 2280
tgaatgttca atgttttaaa tggcgattaa aatactctgc tgtatatagt agtttttgag 2340
taaatatttg caataaaaat ctgccccga ataaaaaaa aaaaaaaa aaaaaaaa 2400
aaaaaaaa aaaaaaaaa aaaaaaaaa aaaaaaaaa aaaaaaaaa aaaaaaaaa 2460
aaaaaaaa aaaaaaaaa aaaaaaa 2487

```

&lt;210&gt; 8

&lt;211&gt; 317

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 8

```

Met Met Ser Phe His Ser Asn Arg Pro Ser Lys Arg Phe Cys Ile Phe
 1              5              10              15

Lys Lys His Ser Glu Asn Leu Arg Gly Ile Thr Leu Val Cys Leu Asn
 20              25              30

Cys Asp Phe Leu Ser Asp Val Ser Gly Leu Asp Asn Met Ala Thr His
 35              40              45

Leu Ser Gln His Lys Thr His Thr Cys Gln Val Val Met Gln Lys Val
 50              55              60

Ser Val Cys Ile Pro Thr Ser Glu His Leu Ser Glu Leu Lys Lys Glu
 65              70              75              80

Ala Pro Ala Lys Glu Gln Glu Pro Val Ser Lys Glu Ile Ala Arg Pro
 85              90              95

Asn Met Ala Glu Arg Glu Thr Glu Thr Ser Asn Ser Glu Ser Lys Gln
100              105              110

Asp Lys Ala Ala Ser Ser Lys Glu Lys Asn Gly Cys Asn Ala Asn Ser
115              120              125

Phe Glu Gly Ser Ser Thr Thr Lys Ser Glu Glu Ser Ile Thr Val Ser
130              135              140

Asp Lys Glu Asn Glu Thr Cys Leu Ala Asp Gln Glu Thr Gly Ser Lys
145              150              155              160

Asn Ile Val Ser Cys Asp Ser Asn Ile Gly Ala Asp Lys Val Glu Lys
165              170              175

Lys Lys Gln Ile Gln His Val Cys Gln Glu Met Glu Leu Lys Met Cys
180              185              190

Gln Ser Ser Glu Asn Ile Ile Leu Ser Asp Gln Ile Lys Asp His Asn
195              200              205

Ser Ser Glu Ala Arg Phe Ser Ser Lys Asn Ile Lys Asp Leu Arg Leu
210              215              220

Ala Ser Asp Asn Val Ser Ile Asp Gln Phe Leu Arg Lys Arg His Glu
225              230              235              240

Pro Glu Ser Val Ser Ser Asp Val Ser Glu Gln Gly Ser Ile His Leu
245              250              255

Glu Pro Leu Thr Pro Ser Glu Val Leu Glu Tyr Glu Ala Thr Glu Ile
260              265              270

Leu Gln Lys Gly Ser Gly Asp Pro Ser Ala Lys Thr Asp Glu Val Val
275              280              285

Ser Asp Gln Thr Asp Asp Ile Pro Gly Gly Asn Asn Pro Ser Thr Thr
290              295              300

Glu Ala Thr Val Asp Leu Glu Asp Glu Lys Glu Arg Ser
305              310              315

```

<210> 9  
 <211> 3495  
 <212> DNA  
 <213> Homo sapiens

<400> 9  
 tttatttttc aaatcataat tttaaaaatga tagataccat tttgtgataa caacaattca 60  
 gaaaacaatt ttctatccctc ttagttgaaa gaatgtaggt acagtttgga tacttggtact 120  
 ttaatttttag agtaaacatc tgcattatac tcttatagat aatagaatta tttagttaag 180  
 aaattcttta cagtaaatga gataatgtgt gaaaaagtat tttgtaaatg ctgaggattc 240  
 tacaaatgat agttgttatt ttcattgtgt tttgtaaatg catgtccatt tcatgaatat 300  
 aggacttcac ataaaaaaag actttctcaa gacaacttta tattctagta tttttctgtt 360  
 gtaaaaagta ttaactatctt acttttatct tgttatacat ttattttaat atccatgtgt 420  
 ttattatagt aaatttgaaa tgaaatcctg aaaaacagaa tttttttaa cacagacctc 480  
 acaccaatat taattttttc tctacataat ttaaaactac ataaattaag tacttaaaat 540  
 ttatattgaa ggccaccaag aacttaggtt gaactctaga aaattttaat aactattttt 600  
 aaagtaccac aacttaatat ttaattttt taatatttat ctctctttac taattcttga 660  
 taaataatag cattagactt gataaaaata aaaagaattt tagagtagaa ttaatatatc 720  
 aaaaggggta tatcaaccaa attggtgtca gattgtattc attctctcat cacataaaga 780  
 tttttctttt gataggtgat gctcatatga acctttggtt tagaatctat atagtacat 840  
 gtgtatgtat gtagatagta tgggtgtata cacacatata taccaaacac catgaatttt 900  
 agcagctctgt gatgatcagc aaaaagcac ataaagtaaa attagttgac catgctaaat 960  
 tcaattctgg aatttttttt tatttgggca tttctagaac tttttacatt tgaagtaca 1020  
 tgatgagtat tagtaacgat gacttatgta taatcagaat ctttatgaca atttagtttt 1080  
 acaaggtcag aagagatgag tttgctaaac ccagctgtga tacctcagtt ggaaagggaa 1140  
 ttcaaaaggta tgctttgttag aacagaaaag tatagttttt ttttcatgaa ctttaatcat 1200  
 tttctgtttt tctctatgt gagtcagcta caaaagtggg ctaattttta caacagtaga 1260  
 acttctctct tttctactgt aatcttccca ctgactttac tgcacaggta tgaataacta 1320  
 gtgtattgga tcttcagtaa cttttttatt tcttagatga ttgaaatata ggtattttact 1380  
 ccatttaaac caggtgataa gatgatgtaa atactcaggg aggggtattaa cttgttactt 1440  
 ttgctcgttt ggggtgtaaa gtgccatgac tgaataatct tcaattcatg attctagagt 1500  
 aagtttaatt tggaaaaaag ggcttcacac atgggtgggtg ttgaacattg attcttttat 1560  
 acttaaaaag atgaaaatgt tttgtggact gatacatttt atcttactga atatgaattg 1620  
 tttatgtatc tctactgtca aatagccttt ttgaaactca ggaagacaa aggttcaatt 1680  
 acaccacttt tgtcaataag caaacagggt attttttttt tctcctgttg tctggatag 1740  
 gcaatagatt ttttaaattg ctgtgagaac ccatatatga aaagagagga gttgaattgt 1800  
 gtgtgccttt tatgtcttga gatttatatg tggaaaagac gacatctact tcaaactgta 1860  
 ttttttctgt tttttttttt tttttgggga aggggggaga acgggggtctt gctctgtcgc 1920  
 ccaggctgga gtgcagtggt gcgactctag ctgactgcaa cctccacctc ccgggttcaa 1980  
 gggattctgc ctgagcctcc cgagtagctg agaccacagg tgcgtgccac cacacccggc 2040  
 taattttttt gtatttttag tagagacggg gtttagtaga gacggatcac tcttgaccac 2100  
 gtgatccgcc cactcgggcc tcccaaagtg ctgggattac aggcgtgagc caccaccccc 2160  
 ggcctgtatt ttcagagagg agagcttggg gtttttggg tgccaagtgg taagataatg 2220  
 tctctttgag gcttctatg gactgccttt atttttagta actcaagaca ccagttaacc 2280  
 tcaacagagt tttggcctta ttagaatttg ttgtgcatct tattgaaagc caggtttaca 2340  
 tcaactcacc ccattattct ttttagttaa ataaatttac catgccaagt aaccagaatg 2400  
 gagcaaattg gttgatcttt aaggcagtag gtttgactag ctatgctatca ttattgtcac 2460  
 atctaattgt aggcaccaga aaccatttga gccaggagtg tgaatgaata attccagag 2520  
 acacttttag cattttttta tgttttata gacattttac atttgggtga ttgccttaga 2580  
 tattaaattt tcttagtgct gataaaaaca gcaacattca taacttattt tatatattgt 2640  
 tccaaagaaa agaatttgtt ttaattggtt caaaataact gcacctgaat ttgtttatgt 2700  
 gccttaagtt ctctagtgtc atttcaactt ttttttcaat ctaaatgaag cttaccttag 2760  
 ataagggtca tatttgttct ctatagagta aataaacttc cccttcttaa attgtgtaat 2820  
 aagcaccacac gtgtgggtgc ttggcagaat gagaatgtta agggagattg ttggatgttt 2880  
 ggagtttcat tatatttttt gtttttattt tttgatacct aggtgctttt taaaatattc 2940  
 agacaaatat ctatcttaca ttgattaaac ccgtgtaaat tcatttgcag tatctacac 3000  
 gaatgtcaaa aaagtatact tatttttggc ccatacttat gtacaatttt tccctcttc 3060  
 aggcctttttc atttaccttt ttgaaaaagc acttactctc cccttcccta tcacccttc 3120

```

cccaagggttt ctttatttaa atttttattg agagttgttg gagctctaag acaatacaaaa 3180
tttagagttg aacaaaagta taatctgctt tacaactagt atagacctaa ggtcatttgc 3240
tttcaattag aggtccaga gtcttcatag tggaaagaat gctttgtatt taattgttct 3300
tagttaagtt gtagcacgtg aatacttact tacatgtttt gtttaaatat acttcttgca 3360
tagtttaatt ttttaaaagt tgtatctaataaaaatgtctt ttaaccatta ttacttgact 3420
atatgggtgt attaaatttt gtttacgaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 3480
aaaaaaaaaa aaaaaa 3495

```

&lt;210&gt; 10

&lt;211&gt; 45

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 10

```

Met Ser Lys Lys Tyr Thr Tyr Phe Cys Ser Ile Leu Met Tyr Asn Phe
  1             5             10             15

```

```

Phe Pro Leu Gln Ala Phe Ser Phe Thr Phe Leu Lys Lys His Leu Leu
      20             25             30

```

```

Ser Pro Ser Leu Ser Pro Leu Pro Gln Gly Phe Phe Ile
      35             40             45

```

&lt;210&gt; 11

&lt;211&gt; 1398

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

```

gttgatccat ctgagaaagg gatcatgaac tagacagaat gaacagcctt agaggcacag 60
actcttgaac gggacgggtg tggtatgact agtgcagagt gtttagagat cactcagttt 120
ttaaagactg gcctttatcg tgtctcagtg cagccgaggc agagcctttg aaggatgcga 180
tggtgtcatt cttactaatc tagtccagcc gctgagggtga ctttcaacgg cagaccgtct 240
cctgagcgcc ccaggtagaa tttcaaaagt ctccgggacc attatggcag tcaagtggac 300
gggtgggcat tcttctctg tctctgcct gaatgcaagt aaagaagggc tgctggcttc 360
tggaagcagag ggccggagatc tcacggcctg ggggtgaagat ggaactccat taggacacac 420
gcggttccaa ggggctgatg atgttaccag tgtcttattt tctccctcct gtcccaccaa 480
gctctatgcc tcacatggag aaaccattag tgtactggat gtcagggtccc tcaaagattc 540
cttgaccat tttcatgtga atgaagaaga aatcaattgt ctttcattga atcaaacgga 600
aaactgtctg gcttctgctg acgactctgg ggcaatcaaa atcctagact tggaaaacaa 660
gaaagttatc agatccttga agagacattc caatatctgc tctcagtggt cttttcggcc 720
tcagaggcct cagagccttg tgatcatgtg actggatatg cagggtgatg tgtggagtct 780
tcaaaaagcc cgaccactct ggattacaaa tttacaggag gatgaaacag aagaaatgga 840
aggccacag tcacctgggc agctcttaaa ccctgcccta gccatttcta tctctgtggc 900
ttcgtgtggt aatattttta gttgtggtgc agaagatggt aagggttcgaa tctttcgggt 960
gatgggagtt aagtgtgaac aggaactggg atttaagggc cacacttcag gggatccca 1020
ggctctgctt ctcccagaat cctatttgcg gcttactgga gggaatgat ggaagatcac 1080
gttggtggat gcaaacagtg aagttgagaa aaaacagaag agtcccacaa aacgtaccca 1140
caggaagaaa cctaaaagag gaacttgcac caagcagggt ggaaatacta acgcttcagt 1200
aacagatgag gaagaacatg gcaacatttt accgaagcta aatattgaac atggagaaaa 1260
agtgaactgg ctcttggtga caaaaataaaa gggacaccaa aatatattag tagctgatca 1320
aactagtgtg atatctgtat accccttaaa tgaattttta atccaataaa aacatttgaa 1380
gaaaaaaaaa aaaaaaaa 1398

```

&lt;210&gt; 12

&lt;211&gt; 358

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 12

```

Met Ala Val Lys Trp Thr Gly Gly His Ser Ser Pro Val Leu Cys Leu
 1           5           10           15

Asn Ala Ser Lys Glu Gly Leu Leu Ala Ser Gly Ala Glu Gly Gly Asp
 20           25           30

Leu Thr Ala Trp Gly Glu Asp Gly Thr Pro Leu Gly His Thr Arg Phe
 35           40           45

Gln Gly Ala Asp Asp Val Thr Ser Val Leu Phe Ser Pro Ser Cys Pro
 50           55           60

Thr Lys Leu Tyr Ala Ser His Gly Glu Thr Ile Ser Val Leu Asp Val
 65           70           75           80

Arg Ser Leu Lys Asp Ser Leu Asp His Phe His Val Asn Glu Glu Glu
 85           90           95

Ile Asn Cys Leu Ser Leu Asn Gln Thr Glu Asn Leu Leu Ala Ser Ala
100           105           110

Asp Asp Ser Gly Ala Ile Lys Ile Leu Asp Leu Glu Asn Lys Lys Val
115           120           125

Ile Arg Ser Leu Lys Arg His Ser Asn Ile Cys Ser Ser Val Ala Phe
130           135           140

Arg Pro Gln Arg Pro Gln Ser Leu Val Ser Cys Gly Leu Asp Met Gln
145           150           155           160

Val Met Leu Trp Ser Leu Gln Lys Ala Arg Pro Leu Trp Ile Thr Asn
165           170           175

Leu Gln Glu Asp Glu Thr Glu Glu Met Glu Gly Pro Gln Ser Pro Gly
180           185           190

Gln Leu Leu Asn Pro Ala Leu Ala His Ser Ile Ser Val Ala Ser Cys
195           200           205

Gly Asn Ile Phe Ser Cys Gly Ala Glu Asp Gly Lys Val Arg Ile Phe
210           215           220

Arg Val Met Gly Val Lys Cys Glu Gln Glu Leu Gly Phe Lys Gly His
225           230           235           240

Thr Ser Gly Val Ser Gln Val Cys Phe Leu Pro Glu Ser Tyr Leu Leu
245           250           255

Leu Thr Gly Gly Asn Asp Gly Lys Ile Thr Leu Trp Asp Ala Asn Ser
260           265           270

Glu Val Glu Lys Lys Gln Lys Ser Pro Thr Lys Arg Thr His Arg Lys
275           280           285

Lys Pro Lys Arg Gly Thr Cys Thr Lys Gln Gly Gly Asn Thr Asn Ala
290           295           300

Ser Val Thr Asp Glu Glu Glu His Gly Asn Ile Leu Pro Lys Leu Asn
305           310           315           320

```

Ile Glu His Gly Glu Lys Val Asn Trp Leu Leu Gly Thr Lys Ile Lys  
 325 330 335

Gly His Gln Asn Ile Leu Val Ala Asp Gln Thr Ser Cys Ile Ser Val  
 340 345 350

Tyr Pro Leu Asn Glu Phe  
 355

<210> 13

<211> 2132

<212> DNA

<213> Homo sapiens

<400> 13

```

ccggaggtag ctaccacggc ctgtgtcaac gactaaagct ccagtacagc ggcgccctca 60
gacagctggg aggggtggctc tggccgggag cggcgggccg tgagctaccg cgaggaggag 120
cggcgggagc gacctcggcc cggccctgca ctggccgccg ggcaggcgcg acatgagcct 180
ggctctggcat ccgcgggatg ctcccttaagc cccttctccg gctgttaacc tccggggaac 240
ggttgtgacc acaccgacac gtattttaca gataaatcat tcttgcggcg gcgggtcgaa 300
cacgtttatt tattttttat ttctcaaca agcttttacc cagcacctgt ccagtgaac 360
aacttgataa tcgtttcgag gggcgteccg cgggttagga agccactgcc tggcagcttg 420
tggaagcctc atttgcaaag cccccctca gatgttttga agatcgtgac gtcttgtaac 480
tagcagtgtg tgcacagaat cctactcaag gaacgtcttg gccagcgat gcaaagaact 540
gaagtttcaa gctggaagag cctgtattgt cctcacaata gtatagaaga attcaagaga 600
ggagagagag acagcaccga atgaagactg taaaagaaaa gaaggaatgc cagagattga 660
gaaaaatctg caagactagg agggtaaccc agaggaaacc gtcttcaggg cctgtttgct 720
ggctatgcct tcgagaacct ggggatcccg aaaaattagg ggaatttctt cagaagaca 780
atatcagcgt gcattatttc tgtcttatct tatctagtaa gctgcctcag aggggcccagt 840
ccaacagagg ttccatgga ttctgctgct aagacatcaa aaaggaggca gcccgggctt 900
ctaggaagat ctgctttgtg tgcaagaaaa agggagctgc tatcaactgc cagaaggatc 960
agtgcctcag aaacttccat ctgccttggt gccaaagaa ggggtgcctt tcacaatttt 1020
ttggagagta caaatcattt tgtgacaaac atcgcccaac acagaacatc caacatgggc 1080
atgtggggga ggaagctgc atcttatgtt gtgaagactt atccaacag agtggtgaga 1140
acatccagag cccgtgttgt agtcaagcca tctaccaccg caagtgcata cagaaatatg 1200
cccacacatc agcaaagcat ttcttcaaat gtccacagtg taacaatcga aaagagtctc 1260
ctcaagaaat gctgagaatg ggaattcata ttccagacag agatgctgcc tgggaactcg 1320
agccaggggc ttctcagac ttatatcagc gctatcagca ctgtgatgcc cccatctgtc 1380
cgtatgaaca aggcagagac agctttgagg atgaaggag gtggtgcctc attctgtgtg 1440
ctacatgcgg atcccacgga acccagagg actgctctc tcttagattt aacagtaaga 1500
aatgggagtg tgaggagtgt tcacctgctg cagccacaga ctacatacct gaaaactcag 1560
gggacatccc ttgtgcagc agcaccttcc accctgagga acatttctgc agagacaaca 1620
ccttggaaga gaatccgggc ctttcttgga ctgattggcc agaaccttcc ttattagaaa 1680
agccagagtc ctctcgtggc agggaggagc actcctggag gtccaagggt gtcagaatca 1740
ctaacagctg caaaaaatcc aagtaacacc ttctgagtag ctgctgtccc acacaatagg 1800
gtatgaagct gcgctcctcc atcgggtttg gggagggagc actctgggac tgtgagacaa 1860
ggaagcaggg ccagcagtga gactatgagc caagcaaaga gaagtctcag tggagcatga 1920
ggaggggagca gtccagatgc caacaaggaa atgcgtttat ggctacaaga gtgcctctgc 1980
tttctcctcc tctcctccca ccaaggattc ttccacctta atcttgtttt catatgcctc 2040
ttcttacttc acccatgttt gttgttatgc aaataaaggt tttctctccc aaaaaaaaaa 2100
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 2132

```

<210> 14

<211> 381

<212> PRT

<213> Homo sapiens

<400> 14



Met Lys Thr Val Lys Glu Lys Lys Glu Cys Gln Arg Leu Arg Lys Ser  
 1 5 10 15  
 Ala Lys Thr Arg Arg Val Thr Gln Arg Lys Pro Ser Ser Gly Pro Val  
 20 25 30  
 Cys Trp Leu Cys Leu Arg Glu Pro Gly Asp Pro Glu Lys Leu Gly Glu  
 35 40 45  
 Phe Leu Gln Lys Asp Asn Ile Ser Val His Tyr Phe Cys Leu Ile Leu  
 50 55 60  
 Ser Ser Lys Leu Pro Gln Arg Gly Gln Ser Asn Arg Gly Phe His Gly  
 65 70 75 80  
 Phe Leu Pro Glu Asp Ile Lys Lys Glu Ala Ala Arg Ala Ser Arg Lys  
 85 90 95  
 Ile Cys Phe Val Cys Lys Lys Lys Gly Ala Ala Ile Asn Cys Gln Lys  
 100 105 110  
 Asp Gln Cys Leu Arg Asn Phe His Leu Pro Cys Gly Gln Glu Arg Gly  
 115 120 125  
 Cys Leu Ser Gln Phe Phe Gly Glu Tyr Lys Ser Phe Cys Asp Lys His  
 130 135 140  
 Arg Pro Thr Gln Asn Ile Gln His Gly His Val Gly Glu Glu Ser Cys  
 145 150 155 160  
 Ile Leu Cys Cys Glu Asp Leu Ser Gln Gln Ser Val Glu Asn Ile Gln  
 165 170 175  
 Ser Pro Cys Cys Ser Gln Ala Ile Tyr His Arg Lys Cys Ile Gln Lys  
 180 185 190  
 Tyr Ala His Thr Ser Ala Lys His Phe Phe Lys Cys Pro Gln Cys Asn  
 195 200 205  
 Asn Arg Lys Glu Phe Pro Gln Glu Met Leu Arg Met Gly Ile His Ile  
 210 215 220  
 Pro Asp Arg Asp Ala Ala Trp Glu Leu Glu Pro Gly Ala Phe Ser Asp  
 225 230 235 240  
 Leu Tyr Gln Arg Tyr Gln His Cys Asp Ala Pro Ile Cys Pro Tyr Glu  
 245 250 255  
 Gln Gly Arg Asp Ser Phe Glu Asp Glu Gly Arg Trp Cys Leu Ile Leu  
 260 265 270  
 Cys Ala Thr Cys Gly Ser His Gly Thr His Arg Asp Cys Ser Ser Leu  
 275 280 285  
 Arg Phe Asn Ser Lys Lys Trp Glu Cys Glu Glu Cys Ser Pro Ala Ala  
 290 295 300  
 Ala Thr Asp Tyr Ile Pro Glu Asn Ser Gly Asp Ile Pro Cys Cys Ser  
 305 310 315 320

Ser Thr Phe His Pro Glu Glu His Phe Cys Arg Asp Asn Thr Leu Glu  
325 330 335

Glu Asn Pro Gly Leu Ser Trp Thr Asp Trp Pro Glu Pro Ser Leu Leu  
340 345 350

Glu Lys Pro Glu Ser Ser Arg Gly Arg Arg Ser Tyr Ser Trp Arg Ser  
355 360 365

Lys Gly Val Arg Ile Thr Asn Ser Cys Lys Lys Ser Lys  
370 375 380

<210> 15

<211> 984

<212> DNA

<213> Homo sapiens

<400> 15

```
gtcacgtgga acctcttaat ctcagcatcc ggagctccag gaagggaataa tttcaagtca 60
gatagaattc tatatatacc atttctttgg aaccttcagc cctcaagatt ccaacatcat 120
gacctcagtt tcaacacagt tgtccttagt cctcatgtca ctgcttttgg tgcctgctgt 180
tgtggaagca gtagaagccg gtgatgcaat cgcccttttg ttaggtgtgg ttctcagcat 240
tacaggcatt tgtgcctgct tgggggtata tgcacgaata agaaatggac agatgtgact 300
ttgaaaggcc tactgagtc aacctcacc tgaaaacct tgctgtttag aggcataacc 360
tgagatttgg tgtgtgaaag gttccaagaa tcagtaaata agggagtttc acatttttca 420
ttgtttccat gaaatggcaa caaacataca ttataaatt gaaaaaaaaa tgttttcttt 480
acaacaaata atgcacagaa aaatgcagcc tataatttgc tagttaggta gtcaaagaag 540
taagatggct gaaatttaca taagtaatat ttcataatct tagaattctc tcaaagcatg 600
tgaaatagga agaaggaagt tcttgcccag aatcttagga aatcaccact gttcgggtat 660
aatcactgcc tctggaatcg ttgaggagtc ttttaaatta gattttgttt ttgtgtgtct 720
ccaagttaat attatatata gatatacag agtcaggcaa aaaggaaaac ttttatctct 780
agggaataaa catttagaaa aatgtattca gtgtatctaa tactgaaatg cggaataaaa 840
tttaattgta aaaaaaaact atagacattg acatggaaaa gagatttaat gttttgaaaa 900
aaaaacttta tattaactga gtaacatcct cctgatgaga agtactatat taaatataaa 960
cccattatgt tataaaaaaa aaaa                                     984
```

<210> 16

<211> 59

<212> PRT

<213> Homo sapiens

<400> 16

Met Thr Ser Val Ser Thr Gln Leu Ser Leu Val Leu Met Ser Leu Leu  
1 5 10 15

Leu Val Leu Pro Val Val Glu Ala Val Glu Ala Gly Asp Ala Ile Ala  
20 25 30

Leu Leu Leu Gly Val Val Leu Ser Ile Thr Gly Ile Cys Ala Cys Leu  
35 40 45

Gly Val Tyr Ala Arg Lys Arg Asn Gly Gln Met  
50 55

<210> 17

<211> 28

<212> DNA

<213> Homo sapiens

<400> 17  
gttcctacag cggtgagagt gaaatgtg

28

<210> 18  
<211> 28  
<212> DNA  
<213> Homo sapiens

<400> 18  
ccgatacacc aaagtctcat ccctaaat

28

<210> 19  
<211> 28  
<212> DNA  
<213> Homo sapiens

<400> 19  
gagctagtgt gaaggacaat aagcagaa

28

<210> 20  
<211> 28  
<212> DNA  
<213> Homo sapiens

<400> 20  
gtcgttaaca tcagaactaa cagattca

28

<210> 21  
<211> 28  
<212> DNA  
<213> Homo sapiens

<400> 21  
gagatactgc aaatgaattt acacgggt

28

<210> 22  
<211> 28  
<212> DNA  
<213> Homo sapiens

<400> 22  
gtcacactta actcccatca cccgaaag

28

<210> 23  
<211> 28  
<212> DNA  
<213> Homo sapiens

<400> 23  
tctgaggcac tgatccttct ggcagttg

28

<210> 24  
<211> 28  
<212> DNA  
<213> Homo sapiens

<400> 24  
tgagggtga aggttccaaa gaaatggt

28

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/18724

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : 435/69.1, 252.3, 320.1; 536/23.1, 23.5, 24.31; 530/350; 514/12

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 252.3, 320.1; 536/23.1, 23.5, 24.31; 530/350; 514/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog, APS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database GENBANK on STN, EST Accession No. R91686, HILLIER et al. The WashU-Merck EST Project. 25 August 1995.	1-5, 11
Y	Database GENBANK on STN, EST Accession No. AA033930, HILLIER et al. The WashU-Merck EST Project. 09 May 1997.	1-5, 11
Y	Database GENBANK on STN, Accession No. R76679, HILLIER et al. The WashU-Merck EST Project. 06 June 1995.	1-5, 11

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

22 OCTOBER 1998

Date of mailing of the international search report

30 OCT 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ENRIQUE B. LONGTON

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/18724

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-11

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/US98/18724

**Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)**

The Abstract does not meet the requirements of PCT Rule 8.1. The new Abstract is:

Novel polynucleotides and proteins encoded by or related to SEQ ID NO:1 and SEQ ID NO:2 are disclosed.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/18724

## A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):

C12N 15/00, 1/21, 15/63, 15/11; C07H 21/04, 21/02; C07K 14/00; A61K 38/00

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-11, drawn to polynucleotide and polypeptide sequences encoded by or related to SEQ ID NO:1 and SEQ ID NO:2.  
Group II, claim(s) 12-14, drawn to polynucleotide and polypeptide sequences encoded by or related to SEQ ID NO:3 and SEQ ID NO:4.  
Group III, claim(s) 15-17, drawn to polynucleotide and polypeptide sequences encoded by or related to SEQ ID NO:5 and SEQ ID NO:6.  
Group IV, claim(s) 18-20, drawn to polynucleotide and polypeptide sequences encoded by or related to SEQ ID NO:7 and SEQ ID NO:8.  
Group V, claim(s) 21-23, drawn to polynucleotide and polypeptide sequences encoded by or related to SEQ ID NO:9 and SEQ ID NO:10.  
Group VI, claim(s) 24-26, drawn to polynucleotide and polypeptide sequences encoded by or related to SEQ ID NO:11 and SEQ ID NO:12.  
Group VII, claim(s) 27-29, drawn to polynucleotide and polypeptide sequences encoded by or related to SEQ ID NO:13 and SEQ ID NO:14.  
Group VIII, claim(s) 30-32, drawn to polynucleotide and polypeptide sequences encoded by or related to SEQ ID NO:15 and SEQ ID NO:16.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The DNA and protein sequences of each of the separate groups are not related to each other structurally or functionally except by virtue of the fact that they encode secreted proteins. Therefore, the inventions do not relate to a single inventive concept under PCT Rule 13.1.